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PHYTOCHEMICAL ANAYLSIS AND ANTIMICROBIAL ACTIVITY OF SELECTED HERBS AGAINST BACTERIAL SEPSIS CAUSATIVE AGENTS

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ABSTRACT
Plants such as *Carum copticum* (Omam) seed extract, *Tagetes erecta* (Samanthi) flower extract, leaf extract of *Centella asiatica* (Vallarai), *Achyranthes aspera* (Nayuruvi) and stem extract of *Aloe vera* (Sothu Kathalai) were the herbs selected for the current study. The study clearly shows that the five herbs could very well be used for the control and treatment of microbial induced septicemia caused by the test organisms of this study such as *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli* and *Pseudomonas aeruginosa* in the Wistar Rats. Though they are found to be effective, their effectiveness is lesser compared to the control antibacterial agents used in this study. They can be used as a supportive agent as folklore medicine, since they are cheaper, side effect free and non-resistant inducing. However further controlled studies are need to be carried out before the usage of these herbal preparation for treating human bacterial septicemic conditions.

KEYWORDS: Bacterial Sepsis, Antimicrobial Activity, Phytochemical Anaylsis**,** *Carum copticum*, *Tagetes erecta*, *Centella asiatica*, *Achyranthes aspera, Aloe vera.*

INTRODUCTION

Sepsis is a complex and heterogeneous syndrome defined as a common inflammatory response to infection (Abraham et al., 2000). It represents a very severe reaction of the immune system, activation of the pro-inflammatory cascades and the compensatory anti-inflammatory response (Van der Poll, 2001). The resulting hemodynamic changes, microcirculatory disturbances and cellular disorders create a disparity between tissue perfusion and metabolic demands. The combination of these factors causes development of multiple organ dysfunction and death (Giantomasso et al., 2003).

Sepsis has been recognized since antiquity (Bone et al., 1992) and is currently the leading cause of death among critically ill adults and the 10th most common cause of death in the United States (Levy et al., 2003; Martin et al., 2003). The incidence of sepsis increased from 164,000 cases (82.7 cases per 100,000 population) identified in 1979 to nearly 660,000 (240.4 cases per 100,000 population) in 2000 (Minino et al., 2004). Certain vulnerable sub-populations, such as people older than 65, neonates and infants, immunocompromised individuals, and critically ill patients, are reported to be at a 1.8 to 65 fold increased risk of developing sepsis (Balk, 2000; Balk et al., 2001; Dremsizov et al., 2004; O'Brien et al., 2007). Despite advances in the care of septic patients, the mortality rates for sepsis in United States have remained high and range from 15% for uncomplicated sepsis to 60% for septic shock (Angus et al., 2001 and Wenzel, 2002).

Every year an estimated 18 million people worldwide developed sepsis with an approximate 30% death rate, which makes it a serious healthcare and social problem (Slade et al., 2003). Research of sepsis in humans is difficult due to the complexity of pathologic processes, heterogeneity of the affected population, lack of firmly established diagnostic markers and restrictions of methodological and ethical nature (Cohen et al., 2001). Taking into account the above difficulties, sepsis models in animals have been created which are affordable and valuable research tools (Fink et al., 1990). They provide a unique opportunity to elucidate the mechanisms of the disease and outline the capacity and specific approaches of therapeutic intervention.

Microbial infection and diseases

Microorganisms often enter the blood stream as a severe complication of primarily localized infections (e.g. pneumonia or urinary tract infection), which represent a failure of the host defense to contain the infection. In this way, the presence of microorganisms in the blood stream becomes an indicator of disseminated infection and as such, generally indicates a poorer prognosis than that associated with localized infections. However, microorganisms may also be transiently introduced into

the bloodstream, e.g. through breaks in mucosal membranes, which may or may not lead to clinical symptoms. In the clinical context, bacteremia can therefore be defined as detection of bacteria or fungi in the blood stream, usually by growth in blood cultures, which are considered of etiological significance based on clinical and microbiological assessment (Weinstein et al., 1983; Schonheyder, 2000). This definition implies that blood culture contamination has been ruled out. Microorganisms that are usually considered to be contaminants include coagulase negative staphylococci, *Corynebacterium* sp., *Bacillus* sp. and *Propionobacterium acenes* unless isolated from two or more separate blood culture sets within a short time frame (Weinstein, 2003).

The causative microbial agents are closely associated with the primary focus of infection. For example, mono microbial bacteremia with *E.coli* often originate from the urinary tract, while bacteremia with *Streptococcus pneumonia* commonly has a primary focus in the respiratory tract. In bacteremia patients the most frequent foci of infection include the urinary tract, the respiratory tract, the abdomen and the skin, bones and joints. The focus of infection is normally assessed on the combination of microbiological and clinical findings (Schonheyder, 2000).

Sepsis in humans is linked to the presence of infectious organisms in approximately 50% of cases. This calculation is probably an under estimate due to the fact that by the time patients have been admitted to the intensive care unit (ICU), they are often on ventilator support and on vasopressors to maintain adequate blood pressure and often have already been treated with broad spectrum antibiotics before admission to the ICU, complicating the ability to identify a causative organism. Clinically, sepsis has been classified as: sepsis, severe sepsis (with SIRS), followed by the presence of multi-organ dis function (MOF) and septic shock (Tang et al, 2010). Progression of sepsis may be due to the inability to contain infectious agents, an example being a leak at a surgical anastomotic site in the colon. Sepsis can also progress because of release of PAMPs or DAMPs. Whatever triggered development of sepsis, the ensuing result is development of SIRS, together with tissue buildup of reactive oxygen species (ROS; including superoxide anion (O^- 2), H_2O_2 and myeloperoxidase products of H_2O_2 and the hydroxyl radical (HO.)]. Reactive nitrogen species [RNS; such as NO (nitric oxide) and peroxynitrite anion (ONOO.)] are also produced. ROS and RNS are reactive with proteins, lipids and DNA, forming adducts. ROS can eventually form a variety of products such as exocyclic ethano-DNA adducts with deoxyadenosine or deoxycytidine (Fang, 2004). In the case of DNA, this can ultimately lead to DNA strand breaks, which then activates the repair enzyme, polyadenosine ribose polymerase (PARP). PARP activation can cause substantial depletion of mitochondrial ATP (Angus, 2010) resulting in defective mitochondrial transfer of electrons and contributing to the buildup of ROS.

Bacteremia

Bacteremia is defined as the presence of viable bacteria in the blood stream (Weinstein et al., 1983). By convention, fungemia (the presence of fungi in the bloodstream) is included in the collective term bacteremia.

Bacteremia can be classified according to the causative microbial agent, the focus of infection and the place of acquisition. Based on the similarity of the isolated microbial agents, bacteremias are usually categorized into those caused by gram-positive, gram-negative, fungal, or anaerobic microorganisms. Furthermore, polymicrobial bacteremia (more than one microbial agent) is distinguished from mono microbial bacteremia (one microbial agent), as polymicrobial bacteremia is associated with a poor prognosis and because the etiologic agent(s) can be difficult to determine.

Septicemia

Sepsis is caused by an immune response triggered by an infection. The infection is most commonly by bacteria, but can also be by fungi, viruses, or parasites. Sepsis is a critical condition often caused by bacterial infection and is considered a common cause of morbidity and mortality (Raoofi et al., 2008).

Sepsis can be defined as the body response to an infection. An infection is caused by microorganisms or germs (usually bacteria) invading the body and can be limited to a particular body region (e.g., a tooth abscess) or can be widespread in the bloodstream (often called septicemia or blood poisoning).

In the present investigation a detailed study has been made on the control and treatment of septicemia causing organisms in induced rats, using indigenous herbals such as *Carum copticum* (Omam) seed extract, *Tagetes erecta* (Samanthi) flower extract, leaf extract of *Centella asiatica* (Vallarai), *Achyranthes aspera* (Nayuruvi) and stem extract of *Aloe vera* (Sothu Kathalai) and an antibacterial agent Ciprofloxacin.

Following are the objectives addressed in the present investigation:

1. To analyze **phytochemica analysis** of the plant extarcts.
2. To study **antibacterial activity** of the selected plants using in vitro methods such as well method and Minimum inhibitory concentration.

To induce septicemia using selected bacteria such as gram positive organisms *Staphylococcus aureus, Streptococcus pyogenes* and gram negative organisms *Escherichia coli* and *Pseudomonas aeruginosa* in rats.

In the present investigation a detailed study has been made on the control and treatment of septicemia causing organisms in induced rats, using indigenous herbals such as *Carum copticum* (Omam) seed extract, *Tagetes erecta* (Samanthi) flower extract, leaf extract of *Centella asiatica* (Vallarai), *Achyranthes aspera* (Nayuruvi) and stem extract of *Aloe vera* (Sothu Kathalai) and an antibacterial agent Ciprofloxacin.

MATERIALS AND METHODS

Glassware, Chemicals and Reagents

The glass wares used were of Borosil® make. The chemicals used were of Hi-media, Sigma and Merck make. All the chemicals and reagents were of analytical grade or extra pure grade. The stains were procured from Qualigens® and Nice Chemicals

Sterilization of Glass wares

The glass wares (petri plates, pipettes, conical flasks etc.,) were sterilized by standard procedure i.e. by immersing in chromic acid solution followed by washing in clean water then by autoclaving (at 121°C and 15lb pressure for 15 minutes), hot air oven(at 160°C for 2 hours) etc.

Animals

Animals are divided into 3 major groups, each group containing 5 (Albino rats) animals. Adult male *albino* rat of Wistar strain weighing around 220 to 260gms were procured from Tamil Nadu Veterinary and Animal Sciences University, Chennai. The animals were kept in polypropylene cages (four in each cage) at an ambient temperature of 25±2°C and 55-65% relative humidity. A 12 ± 1hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions and were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore, India) and had free access to water. The experiments were designed and conducted in accordance with the institutional animal ethics committee.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Preliminary phytochemical analysis

Quantitative and Qualitative estimation of phytoconstituents

Extraction procedure

The plant's stem was washed with fresh water and dried under shade at room temperature, cut into small pieces and the juice was taken using a mixer grinder. Then this juice (100ml) was mixed with solvents such as methanol (85%), chloroform, Ethyl acetate and hexane extracts for overnight at room temperature (Grouch *et al.,* 1992; Matanjun *et al.,* 2008). The extracts were subjected to phytochemical screening for the presence of amino acids, proteins, saponins, triterpenoids, flavonoids, carbohydrates, alkaloids, phytosterols, glycosidal sugars, protein, tannins, phenols and furanoids using the method of (Harborne 1973). The same method was also followed using Soxhlet extraction procedure.

Soxhlet extraction

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity that is insoluble in solvent. If the desired compound has a high solubility in a solvent, then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermolabile compounds as prolonged heating may lead to degradation of different compounds (Roopashree *et al.,* 2008).

Phytochemical screening

Phytochemical examinations were carried out for all the extracts, as per the standard methods (Roopashree *et al.,* 2008).

DETECTION OF ALKALOIDS

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide).
Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). The formation of a brown / reddish precipitate indicates the presence of alkaloids.

c) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of a red precipitate indicates the presence of alkaloids.

d) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids was confirmed by the formation of a yellow coloured precipitate.

DETECTION OF CARBOHYDRATES

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

b) Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c) Fehling's Test: Filtrates were hydrolyzed with dil. HCl neutralized with alkali and heated with Fehling's A

& B solution. Formation of a red precipitate indicates the presence of reducing sugars.

DETECTION OF GLYCOSIDES

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Modified Borntrager's Test: Extracts were treated with a Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammoniacal layer indicates the presence of ethanol glycosides.

b) Legal's Test: Extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

DETECTION OF SAPONINS

a) Froth Test: Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. A formation of 1 cm layer of foam indicates the presence of saponins.

b) Foam Test: 0.5 gm of the extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

ETECTION OF PHYTOSTEROLS

a) Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

b) Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. The formation of brown ring at the junction indicates the presence of phytosterols.

DETECTION OF PHENOLS

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

DETECTION OF TANNINS

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

DETECTION OF FLAVONOIDS

a) Alkaline Reagent Test: Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on the addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test: Extracts were treated with a few drops of lead acetate solution. Formation of a yellow colour precipitate indicates the presence of flavonoids.

DETECTION OF PROTEINS AND AMINOACIDS

a) Xanthoproteic Test: The extracts were treated with a few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

b) Ninhydrin Test: To the extract, 0.25% w/v Ninhydrin reagent was added and boiled for a few minutes. Formation of blue colour indicates the presence of amino acid.

DETECTION OF DITERPENES

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Dosage Given

Animals are divided into five groups each group consisting of five animals (male Wistar albino rats, 10 weeks old and between 220 and 260 g in body weight) and were treated as follows,

- **Group 1** - No induction and treatment (Routine food)
- **Group 2** - Induction with 0.1ml of $0.5x10^5$ CFU/ml of ***Staphylococcus aureus*** given orally to the rat for 30 days.
- **Group 3** - Induction with 0.1ml of $0.5x10^5$ CFU/ml of ***Streptococcus pyogenes*** given orally to the rat as a single dose
- **Group 4** - Induction with 0.1ml of $0.5x10^5$ CFU/ml of ***Escherichia coli,*** given orally to the rat as a single dose
- **Group 5** - Induction with 0.1ml of $0.5x10^5$ CFU/ml of ***Pseudomonas aeruginosa,*** given orally to the rat as a single dose
- **Group 6,7,8,9, 10 and 11** - 0.1ml of $0.5x10^5$ CFU/ml of ***Staphylococcus aureus*** given orally to the rat as a single dose along with ***Carum copticum, Tagetes erecta, Centella asiatica, Achyranthes aspera, Aloe vera*** and an antibacterial agent **Ciprofloxacin** respectively for 14 days
- **Group 12,13,14, 15, 16 and 17** - 0.1ml of $0.5x10^5$ CFU/ml of ***Streptococcus pyogenes*** given orally to the rat as a single dose along with ***Carum copticum, Tagetes erecta, Centella asiatica, Achyranthes aspera, Aloe vera*** and an antibacterial agent **Ciprofloxacin** respectively for 14 days
- **Group 18,19, 20, 21, 22 and 23** - 0.1ml of $0.5x10^5$ CFU/ml of ***Escherichia coli*** given orally to the rat as a single dose along with ***Carum copticum, Tagetes erecta, Centella asiatica, Achyranthes aspera, Aloe vera*** and an antibacterial agent **Ciprofloxacin** respectively for 14 days
- **Group 24,25, 26, 27,28 and 29** - 0.1ml of $0.5x10^5$ CFU/ml of ***Pseudomonas aeruginosa*** given orally to the rat as a single dose along with ***Carum***

copticum, Tagetes erecta, Centella asiatica, Achyranthes aspera, Aloe vera and an antibacterial agent **Ciprofloxacin** respectively for 14 days.

Control and test group rats were caged separately but housed under similar conditions. Both groups of animals were fed with the same diet and water *ad libitum*. All experimental manipulations were carried out with the animals under diethyl ether anesthesia.

Sample Collection and Preparation

The rats were sacrificed at the end of the experimental period and the venous blood was collected into clean sample bottles. This was allowed to clot and then centrifuged at 3000 rpm for 5 minutes after which the serum was separated and stored frozen until needed for analysis.

The animals were observed for Physical activity, feeding, drinking and changes in weight. Finally blood samples were collected and subjected to haematological analysis. Serum samples were collected from those bloods for biochemical analysis. Organs such as liver, lungs and kidney were taken and subjected to histopathological analysis.

MICROBIOLOGICAL ANALYSIS

Antibacterial assay

Antibacterial analysis was followed using standard agar well diffusion method to study the antibacterial activity of extract of the herbs (Perez *et al*., 1990; Erdemoglu *et al*., 2003; Bagamboula *et al*., 2004). Each bacterial isolate was suspended in Brain Heart Infusion (BHI) broth and diluted to approximately 10^5 Colony Forming Unit (CFU) per mL. They were flood-inoculated onto the surface of BHI agar and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer and 100 µL (50 µL oil in ethanol) of the sample solution were poured into the wells. The plates were incubated for 18 h at 37°C. Antibacterial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Ethanol was used as solvent control. Ciprofloxacin was used as reference antibacterial agent. The tests were carried out in triplicates.

The estimation of the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) were carried out by the broth dilution method (Van der Berghe and Vlietinck, 1991). Dilutions of extract of the herbs 2.0 to 0.075 mg/mL were used. Test bacteria culture was used at the concentration of 10^5 CFU/mL. MIC values were taken as the lowest extract of the herbs concentration that prevents visible bacterial growth after 24h of incubation at 37°C and MBC as the lowest concentration that completely inhibited bacterial growth. Ciprofloxacin was used as a reference antibacterial agent and appropriate controls were also used. Experiments were triplicated.

RESULTS AND DISCUSSION

Preliminary Phytochemical Analysis

In the present investigation a detailed study has been made on the control and treatment of septicemia causing organisms in induced rats, using indigenous herbals such as *Carum copticum* (Omam) seed extract**,** *Tagetes erecta* (Samanthi) flower extract**,** leaf extract of *Centella asiatica* (Vallarai), *Achyranthes aspera* (Nayuruvi) and stem extract of *Aloe vera* (Sothu Kathalai).

Study on the Qualitative analysis of phytoconstituents in different extracts of *Carum copticum*

Carum copticum L. commonly known as "Ajwain" is cultivated in many regions of the world including Iran and India, states of Gujarat and Rajasthan. Traditionally, *C. copticum* has been used in the past for various therapeutic effects including bloating, fatigue, diarrhea, abdominal tumors, abdominal pain, respiratory distress and loss of appetite. It has other health benefits such as antifungal, antioxidant, antibacterial, antiparasitic, and hypolipidemic effects (Mohammad Hossein Boskabady et al., 2014).

The qualitative analysis of phytoconstituents in different extracts of *Carum copticum* was carried out using solvents such as Hexane, Chloroform, Ethyl acetate and Methanol. Hexane and Chloroform extract showed alkaloids and Phytosterols. Flavonoids, Polyphenolics, Phytosterol, Carbohydrates, Amino acids and proteins were found to be present in Ethyl acetate and Methanol extract but not in others. Alkaloids, flavonoids and Polyphenols are known to have antimicrobial, antioxidant and immunomodulatory activity. The results are presented in Table No.1.

Table 1- Qualitative analysis of phytoconstituents in different extracts of *Carum -copticum*

Phyto constituents	Hexane	Chloroform	Ethyl acetate	85% Methanol
Alkaloids	+	+	-	-
Flavonoids	-	-	+	+
Polyphenolics	-	-	+	+
Phytosterol	+	+	+	+
Saponins	-	-	-	-
Fixed oils and fats	-	-	-	-
Carbohydrates	-	-	+	+
Amino acids and proteins	-	-	+	+

Mohammad Hossein Boskabady et al., (2014) has reported that *C. copticum* plant contains different important components such as carbohydrates, glucosides, saponins and phenolic compounds (carvacrol), volatile oils (thymol), terpiene, paracymene an betapinene, protein, fat, fiber and minerals including calcium, phosphorus, iron and nicotinic acid (niacin).

Zarshenas et al., (2013) has reported that the constituents of the seed of *C. copticum* includes carbohydrates (38.6%), fat (18.1%), protein (15.4%), fiber (11.9%), tannins, glycosides, moisture (8.9%), saponins, flavone and mineral matter (7.1%) containing calcium, phosphorous, iron, cobalt, copper, iodine, manganese, thiamine, riboflavin and nicotinic acid. Similar results were also reported by Bairwa et al., (2012).

C. copticum grows in different areas of the world containing different compounds. Main components of the oil of Iranian and African *C. copticum* oil are carvacrol, γ- terpinene and p-cymene while thymol (97.9%) is the main component of south Indian plant oil. It was also reported that thymol (45.9%), γ-terpinene (20.6%) and o-cymene (19%) are the major components of the oil of *C. copticum* but ethylene methacrylate (6.9%), β-pinene (1.9%) and hexadecane (1.1%) were the other constituents of the plant. Thymol (72.3%), terpinolene (13.12%) and o-cymene (11.97%) were also identified as constituents of *C. copticum* (Mahboubi and Kazempour, 2011; Kazemi et al., 2011).

Study on the Qualitative analysis of phytoconstituents in different extracts of *Tagetes erecta*

Tagetes erecta, popularly known as marigold, is grown as an ornamental plant. Flowers of this plant are used loose or in garlands for social and religious purposes in Eastern countries. The flowers are usually thrown away after their religious uses. This plant belongs to the family Asteraceae (Compositae). The English name if marigold. Different parts of this plant including flower is used in folk medicine. In traditional and homeopathic medicine it has been used for skin complaints, wounds and burns, conjunctivitis and poor eyesight, menstrual irregularities, varicose veins, hemorrhoids, duodenal ulcers, etc. The flowers are especially employed to cure eye diseases, colds, conjuctivites, coughs, ulcer, bleeding piles and to purify blood (Wichtl, 1994; Manjunath, 1969; Kirtikar and Basu, 1994; Ghani, 2003).

The qualitative analysis of phyto constituents in different extracts of *Tagetes erecta* was carried out using solvents such as Hexane, Chloroform, Ethyl acetate and Methanol. Hexane extract showed alkaloids, Flavonoids, Polyphenolics, Saponins and Phytosterols. Chloroform extract showed alkaloids, and Phytosterols. Alkaloides, Flavonoids, Polyphenolics, saponins and Phytosterols were found to be present in Ethyl acetate extract. Flavonoids, Polyphenolics, Phytosterol, Saponins, Carbohydrates, Amino acids and proteins were identified in 85% Methanol extract. The results are presented in Table No.2.

Table 2- Qualitative analysis of phytoconstituents in different extracts of *Tagetes erecta*

Phytoconstituents	Hexane	Chloroform	Ethyl acetate	85% Methanol
Alkaloids	+	+	-	+
Flavonoids	+	-	+	+
Polyphenolics	+	-	+	+
Phytosterol	+	+	+	+
Saponins	-	-	+	+
Fixed oils and fats	-	-	-	-
Carbohydrates	-	-	-	+
Amino acids and proteins	-	-	-	+

Padalia and Chanda (2015) showed that the extraction ability of different solvents from flower for recovering extractable components followed the order: aqueous > methanol > hexane >acetone > ethyl acetate > toluene. Aqueous extract was superior in their ability to extract phytoconstituents from *T. erecta* (18.11%). Extraction with toluene showed the least yield (0.96%). Methanol and acetone both are polar solvents but methanol had more extractive yield than acetone. Non polar solvent hexane had more extractive yield than polar solvent

acetone while semi polar solvents ethyl acetate and toluene had minimum extractive yield. It was concluded that polar compounds are more than non-polar compounds. Significant differences of extractive yield among different solvents might be attributed to the varied polarity of the solvents. The authors concluded that the choice of solvent has a great influence on the extraction yield but it does not imply that the solvent which had maximum yield will show maximum activity.

Sayema Arefin *et al*., (2015) investigated for proximate analysis, phytochemical screening and antioxidant activity of Bangladeshi marigold (*Tagetes erecta*) flower using Petroleum ether, chloroform, methanol and water extract. The total moisture content, ash value, acid insoluble ash value and water soluble ash value were 11.42%, 7.98%, 4.085% and 3.18% respectively. Water extract exhibited higher extractive value among four extracts. Phytochemical evaluation of methanolic and water extracts confirmed the presence of alkaloids, carbohydrates, saponins, tannins, proteins, flavonoids. Petroleum ether extract revealed the presence of flavonoids. The chloroform extract shows the presence of flavonoids and proteins. The methanolic and water extract shows the presence of alkaloids, reducing sugars, flavonoids, saponins, tannins.

Devika and Justin koilpillai (2012) studied flower's qualitative phytochemical analysis with two solvents such as ethanol and petroleum ether. The bioactive compound, alkaloid was identified promptly in both the solvents. Presence of quinine was observed clearly only with petroleum ether extract of the plant. Presence of triterpenoids was observed in both leaf and flower parts of the plant in petroleum ether extract. Test for coumarin showed a positive result in almost all the parts of the plants in both the solvents except stem (ethanol) and root (petroleum ether). Negative results were obtained for various other phytochemical tests such as saponin, glycoside, terpenoids, steroids and phytosteroids, phlobatanins and anthrox quinones in both the solvent.

Neelapu Neelima *et al.,*(2011) represented the various phytochemicals present in different extracts of marigold flower. The petroleum ether extract contained sterols & terpenoids and fats & fixed oils. The chloroform extract contained glycosides, tannins & phenolic compounds, flavonoids, sterols and terpenoids. The ethyl acetate extract contained alkaloids and flavonoids. The methanol extract contained carbohydrates, proteins & amino acids, steroids & Terpenoids, flavonoids and glycosides. The ethanol extract contained alkaloids, flavonoids and sterols & terpenoids. The aqueous extract contained carbohydrates, sterols & terpenoids and saponins.

Study on the Qualitative analysis of phyto constituents in different extracts of *Centella asiatica*

Centella asiatica L. (Family Apiaceae) is a significant medicinal herb employed based on the familiarity, which is very popular in most tropical and subtropical countries. The whole plant parts are used as medicinal values. The scientific studies have proved a variety of biochemical components i.e. secondary metabolites have been found in *Centella asiatica.* The chemical constituents of *Centella* plant have a very important role in medicinal and neutraceutical applications and it is believed due to its biologically active components of triterpene, saponin. The leaves are extensively utilized as a blood purifier, memory enhancement and for treating elevated blood pressure and prevent ageing. It is one of the chief herbs for treating skin problems, to heal wounds, for revitalizing the nerves and brain cells, hence primarily known as "Brain food" in India (Sharmila Banu Gani et al., 2016; Gohil et al., 2010; Singh and Singh, 2002; Singh et al., 2010; Loiseau and Mercier, 2000).

The qualitative analysis of phytoconstituents in different extracts of *Centella asiatica* was carried out using solvents such as Hexane, Chloroform, Ethyl acetate and Methanol. Hexane extract showed alkaloids and Phytosterols, Saponins, Carbohydrates, Amino acids and proteins. Chloroform extract showed alkaloids, Polyphenolics, Phytosterol. Flavonoids, Polyphenolics, Phytosterol were found to be present in Ethyl acetate extract. Methanol extract showed Alkaloids, Flavonoids, Polyphenolics, Phytosterol, Saponins, Carbohydrates, Amino acids and proteins. The results are presented in Table No.3.

Table 3 - Qualitative analysis of phytoconstituents in different extracts of *Centella asiatica*

Phytoconstituents	Hexane	Chloroform	Ethyl acetate	85% Methanol
Alkaloids	+	+	-	+
Flavonoids	-	-	+	+
Polyphenolics	-	+	+	+
Phytosterol	+	+	+	+
Saponins	+	-	-	+
Fixed oils and fats	-	-	-	-
Carbohydrates	+	-	-	+
Amino acids and proteins	+	-	-	+

Sharmila Banu Gani et al., (2016) the preliminary qualitative and quantitative phytochemical constituents in methanol, hexane and aqueous extracts of *Centella asiatica* L. The qualitative preliminary phytochemical

screening of methanol, hexane and aqueous extracts showed the presence of alkaloids, saponins, Total phenols and tannins, steroids and absence of remaining secondary metabolites.

The phytochemical screening of methanolic extracts of *Centella* are shown by Desai et al., (2013). The presence of some of the phytochemicals has been reported earlier (Singh et al 2012, Arumugam et al 2011). However, it was observed by Arumugam et al 2011) that methanolic extract of leaf indicated presence of alkaloids, steroids, tannins flavanoids, reducing sugars and absence of saponins whereas Sing et al 2012 observed the presence of saponins, alkaloids, glycoside, reducing sugar and absence of flavanoid and tannins. Leaf methanolic extracts of plant indicates presence of almost all phytochemicals except for sugars, tannins, flavones and coumarins (Samy et al., 2011).

Biradar and Rachetti (2016) micro propagated *Centella asiatica* plant and their Quantitative determination phyto constituents were carried out for the powdered plant material by various standard methods and found that alkaloid 0.05gm, 0.2 and 0.2 gm in root, stem, leaf respectively, Flavonoids 0.4gm, 0.4gm and 2.1 gm in root, stem, leaf respectively, terpenoids 0.1gm, 0.3gm and 0.9gm in root, stem, leaf resp. and Saponin 0.1gm, 0.3gm and 0.2gm in root, stem, leaf respectively.

Dharmendra Singh et al., (2012) carried out the methanol extraction of *Centella asiatica* plant and the Qualitative phytochemical analysis of the plant confirms the presence of various phytochemicals like alkaloids, flavonoids, tannins, terpenoids, saponin, steroids and proteins, reducing sugars, carbohydrates and cardiac glycosides.

Study on the Qualitative analysis of phyto constituents in different extracts of *Achyranthes aspera*

Achyranthes aspera Linn. (Amaranthaceae) is an important medicinal plant, commonly known as Chirchita in Hindi, an annual, pubescent stiff erect herb, found as weed throughout India, tropical Asia and other parts of the world 6-7. The inflorescences are pale to bright purple, loosely arranged below and compact above in long spikes. The fruits are oblong cylindrical, yellowish brown, smooth and glabrous. *Achyranthes aspera* is widely used for asthmatic cough, snakebite, hydrophobia, urinary calculi, rabies, influenza, piles, bronchitis, diarrhoea, renal dropsy, gonorrhoea and abdominal pain. *A. aspera* is one of the ethno drugs employed by the tribes for the treatment of various disorders of body including inflammation.

The qualitative analysis of phyto constituents in different extracts of *Achyranthes aspera* was carried out using solvents such as Hexane, Chloroform, Ethyl acetate and Methanol. Hexane extract showed alkaloids and Phytosterols, Carbohydrates, Amino acids and proteins. Chloroform extract showed alkaloids and Phytosterol. Flavonoids, Polyphenolics, Phytosterol and saponins were found to be present in Ethyl acetate extract. Methanol extract showed Alkaloids, Flavonoids, Polyphenolics, Phytosterol, Saponins, Carbohydrates, Amino acids and proteins. The results are presented in Table No.4

Table 4- Qualitative analysis of phyto constituents in different extracts of *Achyranthes aspera*

Phytoconstituents	Hexane	Chloroform	Ethyl acetate	85% Methanol
Alkaloids	+	+	-	+
Flavonoids	-	-	+	+
Polyphenolics	-	-	+	+
Phytosterol	+	+	+	+
Saponins	-	-	+	+
Fixed oils and fats	-	-	-	-
Carbohydrates	+	-	-	+
Amino acids and proteins	+	-	-	+

Dhale and Bhoi (2013) performed the phytochemical studies such as qualitative examination were done on the dried powdered material of *Achyranthes aspera* using the following solvents; ethanol, methanol, diethyl ether and chloroform and the extracts were found to contain protein, glycosides, alkaloids, tannins and phenolic compound, steroid reducing sugars and saponin glycosides.

Phytochemical compounds were screened from *Achyranthes aspera* through with qualitative method. The results assigned the Presence of tannins, Saponin, alkaloids, Acid compounds, sterols, flavonoids and resins absence of steroids, Terpenoids and cardiac glycosides, using ethanol as solvent and the presence of flavonoids which responsible for its anti-mutagenic, antioxidant and anti-inflammatory activity (Annadurai *et al.,* 2015).

The plant parts of *A. aspera* were coarsely powdered and extracted with different solvents, viz., petroleum ether, ethyl acetate, methanol and water. The extracts were then subjected to phytochemical screening as per standard methods prescribed in WHO guidelines. *A. aspera* found to be rich in Alkaloids, Anthocyanins, Anthracene Derivatives, Carotenoids, Cardiac glycosides, Coumarins, diterpenoids, Flavonoids, Saponins, Tannins, Triterpenoids and Volatile Oil. *A. aspera* Leaf found to have Alkaloids, Cardiac Glycosides, and Coumarins

Flavonoids, polyses, Tannins, Triterpenoids and Volatile oils. *A aspera* fruit found to have Alkaloids, Anthocyanins, Anthracene derivatives Cardiac Glycosides, Carotenoids, Coumarins, Flavonoids, Polyses, Saponins, Steroids, Triterpenoids and Volatile Oil. *A. aspera* root detected presence of Alkaloids, Anthracene Derivatives, Lignin, Coumarin, Polyses, Saponins, Steroids, Triterpenoids and Volatile Oil. A *aspera* Stem detected presence of Alkaloids, Anthocyanins, Anthracene Derivatives Lignin, Carotenoids, Cardiac Glycosides, Coumarins Polyses, Saponins, Steroids Triterpenoids and Volatile Oil. qualitative chemical analysis on *A. aspera* revealed it is good source of alkaloids, Anthracene Derivative, cardiac glycosides Diterpenoids, Phenolic Acids and polyphenols, Saponines & Sapogenins, steroids, Sequiterpene Lactones, Triterpenoids and flavonoids (Sonali *et al.,*2015).

In the study by Nisreen Husain and Anil Kumar (2016), all the seven groups of the important phyto compounds, viz., Cardiac glycosides, Steroids, Alkaloids, Flavonoids, Terpenoids, Tannins and Saponins, were screened from the different parts of the well known medicinal herb, *Achyranthes aspera*. Their presence was revealed through in vitro phytochemical screening of methanolic and chloroformic extracts of flower, leaf and root of *Achyranthes aspera*. The analysis for the same was carried out by qualitative tests for which the standard protocol was followed. Methanolic flower and root extracts were found as the good sources of phyto active constituents. Both methanolic and chloroformic extracts of flowers, leaf and root showed the distinct presence of Steroids. Flower and root parts ascertained the presence of Terpenoids. Methanolic extracts of three of the plant parts were found to consist of good stores of phytochemicals as compared to the chloroformic extracts.

Study on the Qualitative analysis of phyto constituents in different extracts of *Aloe vera*

Aloe Vera is a member of Liliaceae family. *Aloe Vera* (L) Burm. Fil (synonym *A. brobadensis* Miller) (Tamil – Southu kathalai; Hindi – Gikanvar) is a cactus like plant with green, dagger – shaped leaves that are flesh, tapering, spiny, marginated and filled with a clear viscous gel. The name was derived from aeabic "alloeh" meaning "bitter" because of bitter liquid found in the leaves. It is present in the arid regions of India and is believed to be effective in treating stomach ailments, skin diseases, anti-inflammatory, wound healing, anti ulcer and diabetes. Currently the plant is widely used in skin care, cosmetics and as nutraceutical (Manikandan and Muhammad Ilyas, 2014). Aloe vera is a unique plant which is a rich source of many chemical compounds and plays an important role in the international market. Chemistry of the plant revealed the presence of more than 200 different biologically active substances including vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, lignin, saponins, sterols, amino acids and salicylic acid (Priyanka Das and Alok Kumar Srivastav, 2015).

The qualitative analysis of phyto constituents in different extracts of *Aloe vera* was carried out using solvents such as Hexane, Chloroform, Ethyl acetate and Methanol. Hexane extract showed Polyphenolics, Phytosterols and Saponins. Chloroform extract showed Flavonoids, Polyphenolics and Phytosterol. The similar chemicals were found to be present in Ethyl acetate extract also. Methanol extract showed Flavonoids, Polyphenolics, Phytosterol, Saponins, Fixed oils and fats, Carbohydrates, Amino acids and proteins. The results are presented in Table No.5.

Table 5- Qualitative analysis of phytoconstituents in different extracts of *Aloe vera*

Phytoconstituents	Hexane	Chloroform	Ethyl acetate	85% Methanol
Alkaloids	-	-	-	-
Flavonoids	-	+	+	+
Polyphenolics	+	+	+	+
Phytosterol	+	+	+	+
Saponins	+	-	-	+
Fixed oils and fats	-	-	-	-
Carbohydrates	-	-	-	+
Amino acids and proteins	-	-	-	+

Aloe vera is reported to contain mono and polysaccharides, tannins, sterols, Organic acids, enzymes, saponins, vitamins and minerals using Distilled water, ethanol and chloroform solutions by Newell et al., (1996).

Priyanka Das and Alok Kumar Srivastav (2015) also carried out the phytochemical analysis of *Aloe vera* .The different solvents such as methanol, petroleum ether, chloroform and aqueous were used to extract the bioactive compounds from the leaves of *Aloe vera*. The detected phytochemical constituents were Tannins, Saponins, Alkaloids, Phenols, Terpenoids and Flavonoids.

The ethanol, methanol, acetone, petroleum ether and aqueous extracts of aloe vera revealed the presence of phytochemicals like tannins, Phlobatannins, saponnin, Flavonoides, terpenoids, cardiac glycosides. Anthraquinone contain phenolic compounds which are

found exclusively in the plant sap. The important are aloes, aloin, aloe-emodin and barbaloin, act as a painkillers. They also function as antibacterial and antiviral (Karpagam and Aruna Devaraj, 2011).

In phytochemical studies by Mariappan and Shanthi (2012), the ethanolic leaf extracts of *Aloe vera* were analysed for the flavonoids, pholabatannis, glycosides, phenols, catachol, resins, saponins, lipids and fats, tannis, acidic compounds, terpenoids, reducing sugars, anthraquinone, carbohydrates, steroids and sterols etc. In analysis of Tannin compounds brownish green colour developed to indicate the presence of Tannin. In this screening process Tannin, Saponin, Flavonoids and Terpenoids compounds revealed positive results.

ANTIBACTERIAL ACTIVITY

The antibacterial activity of the selected five herbs, such as *Carum copticum* (Omam) seed extract**,** *Tagetes erecta* (Samanthi) flower extract**,** leaf extract of *Centella asiatica* (Vallarai), *Achyranthes aspera* (Nayuruvi) and stem extract of *Aloe vera* (Sothu Kathalai), were tested using selected bacteria such as gram positive organisms (*Staphylococcus aureus, Streptococcus pyogenes,*) and gram negative organisms (*Escherichia coli and, Pseudomonas aeruginosa*). Ciprofloxacin was used as antibiotic control.

The antibacterial activity was initially screened by the zone of inhibition by the herbal extracts against the test organism and the results are revealed in Table 20. The antibiotic showed inhibition zone against all the test organisms. Similar was the results for herbals, but the diameter of inhibition zone varied in size for the herbs.

Carum copticum (Omam) seed extract was effective against *Streptococcus pyogenes* and *Escherichia coli* as these organisms exhibited maximum inhibition zone (11mm) followed by *Staphylococcus aureus* (10 mm) while the *Pseudomonas aeruginosa* gave least inhibition zone (7mm). *Tagetes erecta* (Samanthi) flower extract revealed maximum inhibition zone for *Streptococcus pyogenes* (10 mm) while *Staphylococcus aureus* and *Escherichia coli* exhibited 8 mm zone of inhibition and *Pseudomonas aeruginosa* showed 9 mm zone. The leaf extract of *Centella asiatica* (Vallarai), gave best results for *Escherichia coli* (9 mm) and least for *Staphylococcus aureus* (6 mm), while the other two test organisms showed moderate zone (8 mm) of inhibition. The *Achyranthes aspera* (Nayuruvi) extracts exhibited the inhibition zones in the following order *Escherichia coli* (12 mm), *Staphylococcus aureus* (11 mm), *Streptococcus pyogenes* (10 mm) and *Pseudomonas aeruginosa* (7 mm). The stem extract of *Aloe vera* (Sothu Kathalai), showed the inhibition zones in the following descending order viz., *Streptococcus pyogenes* (11 mm), *Staphylococcus aureus* (9 mm), *Pseudomonas aeruginosa* (9 mm) and *Escherichia coli* (8 mm).

The Minimum Inhibitory concentrations (MIC) for the herbs under study on the selected microbes were also carried out. The results for MIC are given in table 13. The antibiotic control had a MIC of 0.25 whereas all the herbs under study showed significance MIC in the range from 1mm to 2 mm. *Centella asiatica* and *Achyranthes aspera* showed a maxium MIC of 2 mm against all four test organisms. *Aloe vera* exhibited a MIC of 1.50 mm for three test organisms (*Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli*) while *Pseudomonas aeruginosa* showed 2 mm MIC. *Carum copticum* gave 1.50mm MIC for *Streptococcus pyogenes* & *Escherichia coli* while for the other two organisms it was 1 mm (*Staphylococcus aureus*) and 2 mm (*Pseudomonas aeruginosa).* The *Tagetes erecta* showed two ranges of MIC's towards the test organisms in the range of 1.50 (*Staphylococcus aureus)*, 2.0 (*Streptococcus pyogenes, Pseudomonas aeruginosa* and *Escherichia coli*).

The Minimum Bactericidal Concentration (MBC) for the selected herbs towards the test organisms under study showed results similar to that of MIC result and is given in table 6.

Table 6- Antibacterial activity of selected herbs

Plants	Organisms (Zone of inhibition in mm)			
	S.aureus	*S.pyogenes*	*E.coli*	*P.aeruginosa*
Carum copticum	9.66±0.33	11.33±0.31	11.65±0.30	7.33±0.32
Tagetes erecta	8.33±0.31	10.33±0.33	8.33±0.23	8.66±0.34
Centella asiatica	6.33±0.31	8.33±0.32	9.33±0.21	8.00±0.00
Achyranthus aspera	11.34±0.33	10.31±0.32	12.02±0.25	7.33±0.32
Aloe vera	8.66±0.32	11.33±.25	8.00±0.31	9.33±0.33
Ciprofloxacin	20.33±0.33	20.00±0.00	16.33±0.32	14.00±0.00

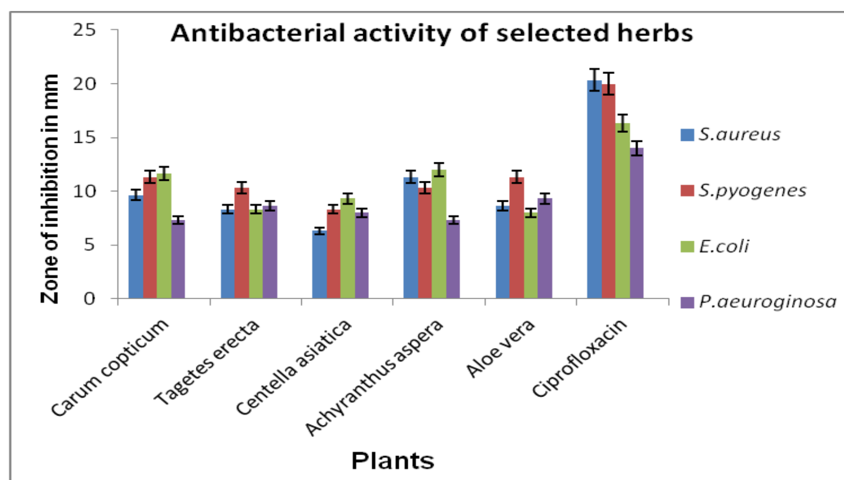

Figure 1- Antibacterial activity of selected herbs

Table 7- MIC against clinical bacterial isolates (mg/ml)

Plants	Organisms (Zone of inhibition in mm)			
	S.aureus	*S.pyogenes*	*E.coli*	*P.aeruginosa*
Carum copticum	1.0	1.5	1.5	2.0
Tagetes erecta	1.5	2.0	2.0	2.0
Centella asiatica	2.0	2.0	2.0	2.0
Achyranthus aspera	2.0	2.0	2.0	2.0
Aloe vera	1.5	1.5	1.5	2.0
Ciprofloxacin	0.25	0.25	0.25	0.25

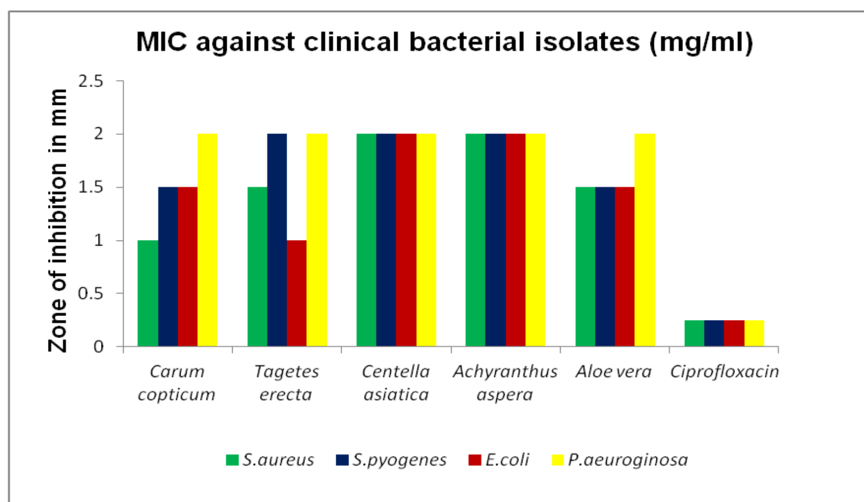

Figure 2- MIC against clinical bacterial isolates (mg/ml)

Table 8- MBC against clinical bacterial isolates (mg/ml)

Plants	Organisms (Zone of inhibition in mm)			
	S.aureus	*S.pyogenes*	*E.coli*	*P.aeruginosa*
Carum copticum	1.0	1.5	1.5	2.0
Tagetes erecta	1.5	2.0	2.0	2.0
Centella asiatica	2.0	2.0	2.0	2.0
Achyranthus aspera	2.0	2.0	2.0	2.0
Aloe vera	1.5	1.5	1.5	2.0
Ciprofloxacin	0.25	0.25	0.25	0.25

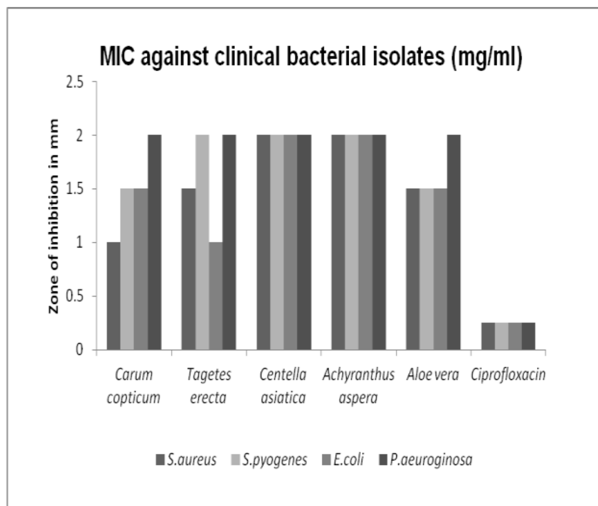

Figure 3- MBC against clinical bacterial isolates (mg/ml)

Figure 4- Antimicrobial activity of plant extracts against *S.aureus*

A- Tagetes erecta B- Carum copticum C- Centella asiatica

B- D- Achyranthus aspera E- Aloe vera

Figure 5-Antimicrobial activity of plant extracts against *E.coli*

A- Tagetes erecta B- Carum copticum C- Centella asiatica

Figure 6- ***MIC of Carum copticum against S.aureus***

The antibacterial activity of omam was studied by Hafiz Muhammad Asif et al., (2014) and they have shown that the Gram positive bacteria such as *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus* and *Listeria monocytogenes* show good inhibition action compared to Gram-negative bacteria (such as *Escherichia coli* and *Pseudomonas aeruginosa*). Gram-negative bacteria generally have been reported to be more resistant than Gram-positive. They have also reported that the antimicrobial activity of omam is due to its phenolic compounds.

Similarly, it was shown that the acetone and aqueous extracts of omam were tested against *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella typhimurium* and *Shigella flexneri* by using agar diffusion assay 16. Methanolic extract of seed of *T. ammi* tested against 11 bacterial species *Pseudomonas aeruginosa* and *Bacillus pumilus*; *Staphylococcus aureus* and *Staphylococcus epidermidis*; *Escherichia coli*, *Klebsiella pneumonia* and *Bordetella bronchiseptica* respectively and showed significant antibacterial activity (Chauhan et al., 2012; Gurinder and Daljit, 2008; Shahidi, 2004).

The effect of aqueous extract of *C. copticum* on several strains of bacteria showed antibacterial effect on *Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, P. aeruginosa, S. typhimurium* and *Shigella flexneri*. The effect of *C. copticum* on fifty-five bacterial strains showed antimicrobial activity with minimum inhibitory concentration <2% (v/v) except *Pseudomonas aeruginosa*. It was also shown that ether fraction of *C. copticum* had better antibacterial and antifungal activity against multidrug resistant (MDR) strains of *Candida albicans, Candida krusei, Candida tropicalis, Candida glabrata, Escherichia coli* and reference strains of *Streptococcus mutans* and *Streptococcus bovis* than other fractions (Mohammad Hossein Boskabady et al., 2014; Kaur and Arora, 2009; Mayaud et al., 2008; Khan et al., 2010).

In the study by Padalia, Chanda (2015), *T. erecta* flowers were extracted with hexane, toluene, ethyl acetate, acetone, methanol and water and tested for Antimicrobial potentiality of the extracts against some 8 Gram positive bacteria, 8 Gram negative bacteria and 4 fungal strains. Aqueous extract showed activity only against *E. aerogenes, P. pseudoalcaligenes* and *P. morganii*. The non-polar solvent hexane inhibited 6 Gram negative bacteria while semi polar solvents toluene and ethyl acetate inhibited 3 and 7 organisms respectively. Maximum inhibition was shown by both polar solvents acetone and methanol. They could inhibit almost all the Gram negative bacterial strains. Acetone extract did not inhibit *E. aerogenes* while methanol extract did not inhibit *P. aerogenosa* and *E. aerogenes*. Highest antibacterial activity was seen against *K. pneumoniae* by all the 5 organic solvents, maximum being by hexane extract. A similar trend was observed against *P. mirabilis* and *P. testosterone*. The non - polar solvent hexane extract showed maximum activity against *K. pneumonia*. The antibacterial activity depends not only on the polarity of the solvent but also on the bacterial strain involved. There are various reports that antibacterial activity depends on the solvent used, structure of the compound in the extracts and the strain under investigation. Different organic solvents extracts have different phytoconstituents in different amounts and that is why there is differential inhibition of the bacteria (Nair et al., 2006).

The antibacterial study of different extracts of leaves and flowers of *Tagetes erecta* Linn. was evaluated according to the agar diffusion method by using gram positive *B.cereus, S. aureus* and gram negative *E.coli, P. aeruginosa*. This study was shown that pet ether extract of leaves and ethyl acetate extract of flower of *Tagetes erecta* significantly inhibit the growth of bacteria dose dependently.

The MIC values of *T. erecta* flowers on Gram negative bacteria were *P. aeruginosa* (156 µg/ml) in fraction 1 and 2, *P. pseudoalcaligenes* (312 µg/ml) in acetone extract, *P. morganii* (625 µg/ml) in fraction 2, *P.mirabilis* (1250 µg/ml) in acetone extract. The MBC values recorded were in the range between 625 µg/ml to >1250 µg/ml. The moderate antibacterial activity was seen against Gram positive bacteria *B. subtilis* (312 µg/ml) in fraction 1, *S. aureus* 2 (625 µg/ml) in acetone extract and its fraction 2, *L. monocytogenes* (156 µg/ml) in acetone extract, *S. albus* (312 µg/ml) acetone extract. The MBC values recorded were in range between 1250 µg/ml to >1250 µg/ml (Padalia Chanda, 2015).

Ullah et al., (2009) was observed the n-hexane, carbon tetrachloride, chloroform soluble fractions of methanol extract from the plant *Centella asiatica* showed antibacterial activity against 5 gram positive bacteria (*Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus* and *Sarcina lutea*) and 8 gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi, Salmonella typhi, Shigella boydii, Shigella dysenteriae, Vibrio mimicus* and *Vibrio parahemolyticus*).

Wei et al., (2008) was observed that methanol extract of *C. asiatica* whole plant showed inhibition zone against *V. alginolyticus, V. vulnificus* and *Streptococcus* sp while inhibition zone was found in *C. freundii* and all *Vibrio* sp. except *V. vulnificus* against aqueous extracted *C. asiatica* whole plant. Taemchuay et al., (2008) was find out that crude extract of *Centella asiatica*, particularly extracted with water, had a promising antibacterial effect against *Staphylococcus aureus*. Water extracts of Centella leaves cultivated in Similipal biosphere reserve in Orissa, India showed antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, Shigella flexneri* and *Candida kruesi* (Thatoi et al., 2008).

Methanolic extract of Centella was showed antibacterial activity against 3 *Vibrio* species named *V. harveyi, V. alginolyticus* and *V. parahaemolyticus*. But acetone, chloroform and hexane cxtracts was not showed antibacterial activity against these species (Sankar et al., 2010). Hexane, dichloromethane, ethyl acetate, diethyl ether and methanol extracts of *Centella asiatica* showed antibacterial activity against *B. subtilis, K. aerogenes, P. vulgaris* and *S. aureus* species. But they did not show antibacterial activity against *Escherichia coli* and *Pseudomonas aerogenes* (Samy and Ignacimuthu, 2000). Similarly Srivastava et al., (1997) and Zaidan et al., (2005) was also observed the antibacterial activity of Centella plant extracts by using diffusion method. Methanolic extract of *C. asiatica* showed antibacterial activities to gram positive *S. aureus* and Methicillin Resistant *S. aureus* (MRSA). But it did not show antibacterial activities to gram negative bacteria like *E. coli* and *K. pneumonia*.

Ethanolic extract of *Centella asiatica* shows significantly higher rate of sensitivity against various bacteria strains like *Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Propionibacterium vulgaris*, while petroleum ether extract shows moderately sensitivity and water extract showed that least sensitivity against these strains. Crude extracts of *Centella asiatica* showed antibacterial activity against bacteria like *Propionibacterium acnes* and *Staphylococcus epidermidis* (Chomnawang et al., 2005).

Panthi and Chaudhary (2006) was observed that methanolic extracts of *Centella asiatica* collected from Nepal showed antibacterial activity against one gram positive bacteria *Staphylococcus aureus* and three gram-negative bacteria like *Escherichia coli, Pseudomonas aeruginosa* and *Shigella boydii*. But another similar study in Nepal by Mahato and Chaudhary (2005) was not observed antibacterial activity in methanolic extracts of *Centella asiatica* against bacteria like *Bacillus subtilis, Staphylococus aureus, Escherichia coli* and

Pseudomonas aeruginosa. The crude extracts from *Achyranthes aspera* were detected to be prominently active against the tested microorganisms at the different concentrations. Among the bacteria *Staphyloccocus aureus* spp. formed high zone of inhibition which showed the *Achyranthes aspera* has high antibacterial activity against *Staphylococcus aureus* spp. The extracts showed antimicrobial activity against both Gram-positive and Gram-negative bacteria (Annadurai et al*.*, 2015).

CONCLUSION

Plants such as *Carum copticum* (Omam) seed extract, *Tagetes erecta* (Samanthi) flower extract, leaf extract of *Centella asiatica* (Vallarai), *Achyranthes aspera* (Nayuruvi) and stem extract of *Aloe vera* (Sothu Kathalai) were the herbs selected for the current study. The study clearly shows that the five herbs could very well be used for the control and treatment of microbial induced septicemia caused by the test organisms of this study such as *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli* and *Pseudomonas aeruginosa* in the Wistar Rats. Though they are found to be effective, their effectiveness is lesser compared to the control antibacterial agents used in this study. They can be used as a supportive agent as folklore medicine, since they are cheaper, side effect free, and non-resistant inducing. However further controlled studies are need to be carried out before the usage of these herbal preparation for treating human bacterial septicemic conditions.

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