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STUDIES ON ALKALOIDS EXTRACTION, IDENTIFICATION BY THIN LAYER CHROMATOGRAPHY FROM SOME MEDICINAL PLANTS AND EVALUATING ITS ANTIMICROBIAL POTENTIAL.

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ABSTRACT
Herbal medicine is the use of medicinal plants for prevention and treatment of diseases: it ranges from traditional and popular medicines of every country to the use of standardized and titrated herbal extracts. It aims to return the body to a state of natural balance, so that it can start healing itself. Different herbs act on different systems of the body. They tend to be more effective for long-standing health complaints that don't respond well to traditional medicine. In the present study, alkaloids extraction and its presence were identified in six medicinal plants (*Anemone obstusiloba, Terminalia chebula, Withania somnifera, Acacia nilotica and Convolvulus pluricaulis) and* Pudina (Mentha sp.) with the help of TLC method. *Convolvulus Pluricaulis* (Shankpushpi) and Mentha sp. (Pudina) showed intense violet colour bands under UV light proving the presence of alkaloids .The present study was further carried out to find out the antibacterial activity of methanolic extract of *Convolvulus pluricaulis* and Mentha sp. against Gram-negative bacteria (*Escherichia coli)* and Gram-positive bacteria (*Staphylococcus aureus*) using agar well diffusion method. Our finding suggest that methanolic extract of *Convolvulus pluricaulis* and Mentha sp.has potent antibacterial activity against the pathogenic strains of *Staphylococcus aureus* and *E.coli* and exhibited the significant wide spectrum of antibacterial activity against both Gram's positive and Gram's negative bacteria. The broad-spectrum antimicrobial activity of these plant extract, possibly due to the reported secondary metabolites, further confirm its use as a health remedy in folklore medicine. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial and fungal infections including gonorrhoea, pneumonia, eye infections and mycotic infections.

KEYWORDS: Medicinal plants, Alkaloids, TLC, Antimicrobial activity.

INTRODUCTION
Plant material have been used for the treatment of serious diseases throughout the world before the advent of modern clinical drugs[1].The use of medicinal plants still plays an important role to cover the basic health needs in the developing countries. Several top selling drugs of modern times such as Qunine, Artemisinin, Shikonin, etc. are obtained from plants1. Most of the phytochemicals, secondary metabolites of plants, are physiological active[2]. Majority of phytochemicals are known to produce therapeutic activities like antibacterial, antifungal, antioxidant etc. Alkaloids, tannins, flavonoids and phenol compounds are the most important of bioactive constituent of plants. In addition to their use for therapeutic purposes, natural phytochemicals are effective as precursors for the synthesis of novel useful drugs. About 50% of modern drugs are natural products, which play an important role in drug development in pharmaceutical industry. The exploitation of plants by man for treatment of diseases has been in practice for a very long time. Medicinal plants are of great value to mankind and society. The medicinal value of these plants can be observed from the chemical agents they possess which may alter certain physiological actions in the human body. The most important of these bioactive constituents of plants are alkaloid; tannins, flavonoids and phenolic compounds[3]. Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to food meant for pregnant and nursing mothers for medicinal purposes[4]. These plants are used as sources of therapeutic agents due to their higher properties. These include among others reduced cost, relative lower incidence of adverse reactions compared to modern synthetic pharmaceuticals[5]. Knowledge of the chemical constituents of medicinal plants are desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of economic materials such as tannins, oils, gums, precursors for the synthesis of complex chemical substances .Plant based

natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds etc.[6] Medicinal and aromatic plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals, perfumery, flavor and cosmetic industries. The plant kingdom has served as an inexhaustible source of useful drugs, foods, additives, flavoring agents, lubricants, coloring agents and gums from time immemorial. The therapeutic power of herbs had been recognized since creation of the universe and botanic medicine is one of the oldest practiced professions by mankind[7]. Medicinal plants have been found useful as antimaleria, antisickling, anti-helminthic, anti-microbial and anti-hypertensive agent's etc.[8]

The aim of the present study is to extract and identify alkaloids from medicinal plants by chromatographic technique and to check its antimicrobial activity against bacteria by agar well diffusion method.

MATERIALS AND METHOD

Collection of samples

The leaves, bark, roots and skin of 5 medicinal plants which were used in this study are in powdered form collected from an Ayurvedic store from local market in Mumbai, Maharashtra. The 5 plants were – Ratan Jot (*Anemone obstusiloba*), Hirda (*Terminalia chebula*), Ashwagandha (Withania somnifera), Shankpushpi (Convulus pluricaulis) and Babool (*Acacia nilotica*).

Leaves of Pudina (Mentha sp.) were taken washed and dried in a hot air oven at 50° C for 24 hours. Then the dried leaves were crushed using a blender and the sample was made.

Solvent Extraction method for secondary metabolites

All the 6 samples powder was weighed 2 gm and mixed with 20ml methanol in a plugged conical flask. The mixture was kept on shaker at room temperature. After 24 hours it was filtered using Whatman filter paper no.1. The filtrate was collected in a beaker and dried in a hot air oven at 50 C for 24 hour. The dried product was weighed and used as a crude extract.

Separation, Identification and Confirmations of Secondary metabolites

Separations of alkaloids from all the 6 medicinal plant extracts were done by the TLC method. The solvent system used for TLC is chloroform: methanol (15:1). Secondary metabolites were identified by using UV light (UV transilluminator) which gives violet colour florescence[9].

Test organisms

The test microorganisms, *Escherichia coli* and *Staphylococcus aureus* were obtained from culture repository of Biotechnology department. The organisms were inoculated onto NB incubated 37˚C for overnight for the growth of the bacteria and prepared the inoculum as 1.0×10^6 cfu/ ml[10].

Preparation of different concentration of crude extract

The stock solutions of 100mg/ml of crude extract of all medicinal plants were prepared. Different concentrations (6.25, 12.5,25 and 50 mg/ml) of stock were prepared by 1:2 dilution method.

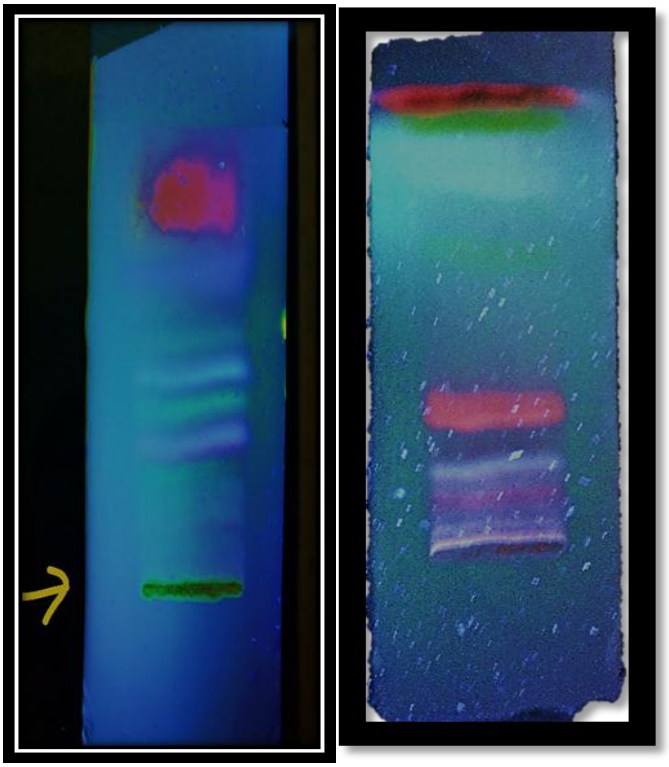
Determination of Antibacterial Activity by agar well diffusion method

The antibacterial activity of the crude extract of the medicinal plant (Shankpushpi and mint leaves) which showed the presence of alkaloids was determined by Agar well diffusion method. Petri dishes (100mm) containing 20ml of Mueller Hinton Agar seeded with 0.2 ml inoculum of bacterial strain. Well of 8mm diameter were cut into solidified agar media with the help of sterile cork-borer. 50 µl of different concentration of extract was poured in the respective well and the plates were kept at 4C for pre diffusion and later incubated at 37C for overnight. Organic solvent (methanol) were used as negative control while Ampoxin antibiotic was used as a positive control. The experiment was performed in duplicate under strict aseptic conditions and the anti-bacterial activity of each extract was expressed in terms of diameter of zone of inhibition (in mm) produced by the respective extract at the end of incubation period[10].

RESULTS AND DISCUSSION

Alkaloid extraction and identification

Alkaloid separation and its presence were detected by TLC method and only 2 plants *Convulus pluricaulis* and Mentha sp. showed confirm results of secondary metabolites as purple colour bands under UV-illuminator.(Fig 1). Our results were found in agreement with some earlier studies in which among 28 medicinal plants only 18 plants gives positive results. Gujpatta, Sadapatta, Shankhapushpi and makka, were given instance bands of secondary[9]. Other report also showed the presence of alkaloids by TLC , five orange bands were observed for *M. citrifolia* fruit, six for *A. squamosa* and five bands for *A. angustiloba*. TLC of alkaloid extracts from the plants used in their study revealed the presence of these compounds by using Dragendroff's reagent to reveal characteristic orange bands of alkaloids. *M. citrifolia*, *A. squamosa* and *A. angustiloba* are known for their alkaloid content.[11]



Convulus pluricaulis **Mentha sp.**

Figure1: TLC plates showing the presence of alkaloids in *Convulus pluricaulis* and Mentha sp

Antimicrobial activity of *Convulus pluricaulis* plant extract

The result of screening of antimicrobial activity of different dilutions of Methanolic extract of *C. pluricaulis* is summarized in Table 1. It is evident from the zone of inhibition (Figure 2) that the methanolic extract exhibited prominent antimicrobial activity against two bacterial strains viz. *E. Coli* and *S. aureus* used in this study. The zone of inhibition of Methanolic extract was found to be in range of 15-26mm against *E. coli* and 23-35mm against *S. aureus*. This was comparable with the standard drug, which showed no inhibition against *E. coli* and 32 mm against *S. aureus* respectively. From the study it was also concluded that *C. pluricaulis* is more active against *S. aureus* in comparison to *E. coli*. Our results were found in agreement with some earlier studies which showed the high antibacterial activity against *S.aureus* and moderate antibacterial properties of shankpushli extract against *E. Coli*[12].The evaluation of antimicrobial potential by disc diffusion method indicated that all the bacterial strains tested showed growth inhibition toward the plant extract, however, with differing sensitivity. Among the bacterial pathogens, *E. coli* is more sensitive compared to *Staphylococcus* and *Acenatobacter*.

Table 1-:Zone of inhibitions of different dilutions of *Convulus pluricaulis* against test organisms.

Name of extract	Dilutions	Zone of inhibition. (mm)	
		Escherichia coli	***Staphylococcus aureus***
***Convulus pluricaulis* (Shankpushpi)**	**100**	**26**	**33**
	50	**22**	**30**
	25	**19**	**28**
	12.50	**18**	**26**
	6.25	**15**	**23**
	Drug.	**Nil**	**32**

Figure 2. Zone of inhibitions of different concentrations of Shankpushpi against*S.aureus* and *E.coli.*

Antimicrobial activity of Pudina *(Mentha sp*.) plant extract

The various crude extracts of Mentha sp. showed significant activity against all the bacteria tested. (Table 2). The antimicrobial activity against *E.coli* is found to be more than *S.aureus* (Fig 3). On overall, leaf extracts are very active against all the tested bacterial strains Antibacterial properties of leaf extracts against these bacteria suggests that these extracts can be used for wound healing and septicaemia. Similar results were also reported by scientist[13] in which they extracted the secondary metabolite and made its mixture in different solvents and tested it against the organisms. They found more antimicrobial activity for *S.aureus* than *E.coli.* The molecular targets of alkaloids extracted from these crude extracts are neuroreceptors and are mutagenic as they intercalate DNA and interfere with DNA, telomeres and telomerase.

Table 2- Zone of different dilutions of *Mentha sp(* mint) aganist test organisms.

Extract	Zone of inhibition. (mm)		
	Concentrations	*S.aureus*	*E.coli*
Mentha sp **(mint leaves)**	100	25	38
	50	23	35
	25	21	31
	12.50	16	28
	6.25	14	23
	Drug	Nil	32

Figure 3 -Zone of inhibitions of different concentrations of menthe sp. extract against test organisms

CONCLUSION

The present study suggested that, the solvent extracts of Mint leaves and *Convolvulus pluricaulis* have a great potential as antimicrobial agents against selected enteric pathogens and they can be used as an alternative medicine in the treatment or control of enteric bacterial infections. TLC analysis showed presence of antimicrobial substances in the studied extracts. The results revealed the presence of medicinally important constituents in these solvent extracts. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Therefore, the Mint leaves and *Convolvulus pluricaulis* solvent extracts could be seen as a good source for useful drugs. Methanolic extract of *Convolvulus pluricaulis* and *Mentha sp*. in this study demonstrated a broad-spectrum of activity against both gram-positive and gram-negative bacteria. The broad-spectrum antimicrobial activity of the plant extract, possibly due to the reported secondary metabolites, further confirm its use as a health remedy in folklore medicine. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial and fungal infections including gonorrhoea, pneumonia, eye infections and mycotic infections. Isolation, identification and purification of these alkaloids and determination of their respective antimicrobial potencies and toxicological evaluation with the view to formulating novel chemotherapeutic agents should be the future direction for investigation.

As the work for the development of herbal medicines is in progress worldwide, the present report will help in the isolation of new products/drugs. Finally, it can be concluded that the active chemical compounds present in Mentha sp. and *Convulus pluricaulis* should certainly find place in treatment of various bacterial infections and indicate this herb should be studied more extensively to explore its potential in the treatment of infectious diseases as well.

Future prospective

Medicinal plants are increasingly gaining acceptance even among the literates in urban settlements, probably due to the increasing inefficacy of many modern drugs used for the control of many infections such as typhoid fever, gonorrhoea, and tuberculosis as well as increase in resistance by several bacteria to various antibiotics and the increasing cost of prescription drugs, for the maintenance of personal health. Current problems associated with the use of antibiotics, increased prevalence of multiple-drug resistant (MDR) strains of a number of pathogenic bacteria such as methicillin resistant Staphylococcus aureus, Helicobacter pylori has revived the interest in plants with antimicrobial properties. Therefore, the use of this alkaloids extracted can be used to cure diseases like (AIDS) and used in

chemotherapy. Secondly, it can be used in monitoring of AST patterns of clinical isolates which can be further useful for the early detection of outbreaks caused by antibiotic-resistant pathogens of clinical and epidemiological relevance.

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