ejpmr, 2015,2(6), 171-176

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

STUDIES ON THE PHYTOCHEMICAL, SPECTROSCOPIC CHARACTERIZATION AND ANTIBACTERIAL EFFICIENCY OF *URGINEA INDICA*(ROXB.) KUNTH (LILIACEAE) AND *CYCLEA PELTATA* ARN. EX WIGHT (MENISPERMACEAE).

[a]U. S. Patil, [b]O.S.Deshmukh and [c*]R.P.Ganorkar.

[a]Department of Botany, Bharatiya Mahavidyalaya, Amravati. (MS)India.
[b]Department of Botany, MahatmaFule Arts, Commerce and Sitaramji Choudhari Science Mahavidyalaya, Warud, Dist. Amravati. 444906, (MS) India.
[c]Department of Chemistry, Mahatma Fule Arts, Commerce and Sitaramji Choudhari Science Mahavidyalaya, Warud, Dist. Amravati. 444906, (MS) India.

***Correspondence for Author: O.S.Deshmukh**
Department of Botany, Mahatma Fule Arts, Commerce and Sitaramji Choudhari Science Mahavidyalaya, Warud, Dist. Amravati. 444906, (MS) India.

Article Received on 23/08/2015 **Article Revised on 15/09/2015** **Article Accepted on 07/10/2015**

ABSTRACT

A large number of medicinal plants are used as alternate medicine for diseases of man and other animals since most of them are without side effects when compared with synthetic drugs. Each plant whether it may be shrub, herbs, algae have its own significance in pharmaceutical, medicinal, agricultural, industrial, biochemical and chemical sciences. The present investigation was focused on the preliminary phytochemical and Fourier Transform Infrared Spectral analysis and Antimicrobial Studies of solvents extracts of *Urginea indica* (Roxb.) Kunth (Liliaceae) and *Cyclea peltata* Arn. ex Wight (Menispermaceae), results were clearly revealed that the plant contained different bioactive compounds such as of Alkaloids, Anthoquinones, Coumarins, Steriods and Flavonoids compounds were rich in the extracts of *Urginea indica* (Liliaceae) and *Cyclea peltata* (Menispermaceae) are connected with defense mechanism against many microorganisms.

KEYWORDS: Antimicrobial, Spectroscopical,Phytochemical, terpenoids, flavonoids, saponins, alkaloids, flavonoids, steroids, *Urginea indica, Cyclea peltata.*

INTRODUCTION

There has been an increasing interest worldwide on therapeutic values of natural products. The nature provides the mankind vast therapeutic flora with a wide variety of medicinal potential. The revival of interest in plant derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs many of which have adverse side effects.

In recent years, phytochemicals in vegetables have received a great deal of attention mainly on their role in preventing diseases caused as a result of oxidative stress which releases reactive oxygen species such as singlet oxygen and various radicals as a damaging side effect of aerobic metabolism. Characterization of extracts of medicinal plants is necessary, due to its numerous benefits to science and society. The information obtained, makes pharmacological studies possible. It also enabled structure-related activity studies to be carried out, leading to the possible synthesis of more potent drug with reduced toxicity. The mode of action of the plants producing the therapeutic effect can also be better investigated if the active ingredients are characterized.

The use of phytochemicals as natural antimicrobial agents, commonly called 'biocides' is gaining popularity.[1] The most essential of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. *Urginea indica* (Roxb.) Kunth *and Cyclea peltata* Arn. ex Wight are an attractive,succulent medicinal plant of the family Liliaceae and Menispermaceae. They are medicinal impotant plants distributed in Uttamsagar, Prabhat pattan and Satpuda hills of Betul District, Madhya Pradesh , India.[2] Plants produce bioactive molecules in a diverse range making them a rich source of different types of medicines.[3-6] Ramamoorthi and Kannan screened the bioactive group of chemicals in the dry leaf powder of *Calotropis gigantea* by FTIR analysis.[7] Kareru *et.al*.detected saponins in crude dry powder of 11 plants using FTIR spectroscopy.[8]

The most essential of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food.[9]Traditionally herbal extracts were known to be effective against microorganisms as a

result; plants form the basis of modern medicine. Plants produce phytochemicals to protect themselves; but recent studies indicate that many phytochemicals can also protect humans against infectious diseases.[10-13] Antimicrobial activity and phytochemical screening of *Urgenia indica* (Jangali Kanda) bulb and Cyclea peltata (Pahadkand) rhizome, in this study suggest that these plant can be used as an antimicrobial agent and expected that these may be used as therapeutic agents for various diseases.[14]

MATERIALS AND METHODS

Plant Collection: *Urginea indica* (Roxb.) Kunth(Liliaceae) and *Cyclea peltata* Arn. ex Wight (Menispermaceae) were collected from Fokala forest area of Betul district, Madhya Pradesh, India. Before picking the plant, the soil was moisted and then shade dried, were ground to get a course powder that was stored in airtight, high density poly ethylene container.

***Urginea indica* –Bulb**

***Cyclea peltata*- Rhizome**

Source and extraction

The organic solvent extract was prepared by adding 5 gm powder of ethno veterinary medicinal plants in 250 ml of organic solvent(Absolute Alcohol, Acetone, Petrolium Ether, Benzene, chloroform and Distil Water) for 6 hrs. by soxlhet method and filtrate was evaporated in controlled conditions of temperature of active constituents of preparations. Dried extracts were stored in labeled sterile wide mouthed screw capped bottle at 4^0c and used for further study.

Phytochemical screening

Phytochemical screening were performed to assess the qualitative chemical composition of different crude extracts using commonly employed precipitation and coloration reactions ,the methods of Harbone[15], Trease and Evans[16] were used to identify the major secondary metabolites like Alkaloids, Flavonoids, Saponins, Carbohydrate, Protein, Phenols, Steroids, Tannins, Glycosides, Terpenoids, Phlobatannins, Coumarins, Emodins, Anthoquinones, Anthocyanins, Leucoanthocyanins in the extracts.

Antimicrobial screening

The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India. The bacteria rejuvenated in Nutrient broth (Hi-media – laboratories, Mumbai, India) at 37^0c for 18 hrs. and then stored at 4^0c on Nutrient agar subcultures were prepared from the stock for bioassay. Bacterial Pathogen used in study are, *E. coli* ,*Pseudomonas fluroscene* ,*Salmonella typhi Bacillus subtilis* ,*Klebsiella pneumoniae* ,*Staphylococcus aureus* ,*Streptococcus* Yeast candida ,*Aspergillus niger*. The disc-diffusion test is based on the fact that for a given sample of plant extract. Antimicrobial susceptibility testing with discs is a simple and rapid method and provides a reproducible means of testing bacterial sensitivity to various antibiotics and chemotherapeutic agents.

Infrared (IR) spectroscopy analysis

This was done using infrared spectrophotometer of Shimadzu Corporation of model IR prestige 21. The extracts were scanned in accordance with ASTM 1252-98. A drop of each extract was applied on a sodium chloride cell to obtain a thin layer.

RESULT AND DISCUSSION

Table 1: Preliminary phytochemical screening of various extracts of *Urginea indica*(Roxb.) Kunth.

Constituents	Test	Petrolium ether extract	Benzene extract	Chloroform extract	Acetone extract	Ethyl Alcohol extract	Distil water extract
Alkaloids	Hager's test	-	-	-	-	+	+
Flavonoids	Lead acetate test(Pb(OAc)4s	-	-	-	-	+	-
Saponins	Foam test	-	-	-	-	-	-
Carbohydrate	Molisch test	-	-	-	-	-	-
Protein	Xanthoproteic test	-	-	-	-	-	-

Phenols	Ferric chloride test	-	-	-	-	-	-
Steroids	Salkowski test	-	-	-	-	-	-
Tannins	Braymer's test	-	-	-	-	-	-
Glycosides	Libermann's test	-	-	-	-	-	-
Terpenoids	Acetic unhydride tset	-	-	-	-	-	-
Phlobatannins	Precitate test	-	-	-	-	-	-
Coumarins	10% NaOH test	+	-	-	-	+	-
Emodins	NH4OH & Benzene test	-	-	-	-	-	-
Anthoquinones	Borntrager's test	+	+	+	+	+	_
Anthocyanins	2N HCL & Ammonia test	-	-	-	-	-	-
Leucoantho-cyanins turns	Isoamyl alcohol test	-	-	-	-	-	-

Present -- +ve Absent -- -ve

Table 2: Preliminary phytochemical screening of various extracts of *Cyclea peltata* Arn. ex Wight

Constituents	**Test**	**Petrolium ether extract**	**Benzene extract**	**Chloroform extract**	**Acetone extract**	**Ethyl Alcohol extract**	**Distil water extract**
Alkaloids	Hager's test	+	+	-	-	+	+
Flavonoids	Lead acetate test(Pb(OAc)4s	+	-	-	-	+	+
Saponins	Foam test	-	-	-	-	-	-
Carbohydrate	Molisch test	-	-	-	-	-	-
Protein	Xanthoproteic test	-	-	-	-	-	-
Phenols	Ferric chloride test	-	-	-	-	-	-
Steroids	Salkowski test	+	+	+	-	-	+
Tannins	Braymer's test	-	-	-	-	-	-
Glycosides	Libermann's test	-	-	-	-	-	-
Terpenoids	Acetic unhydride tset	-	-	-	-	-	-
Phlobatannins	Precitate test	-	-	-	-	-	-
Coumarins	10% NaOH test	+	+	+	+	+	-
Emodins	NH4OH & Benzene test	-	-	-	-	-	-
Anthoquinones	Borntrager's test	-	-	-	-	-	-
Anthocyanins	2N HCL & Ammonia test	-	-	-	-	-	-
Leucoanthocyanins turns	Isoamyl alcohol test	-	-	-	-	-	-

Present -- +ve Absent -- -ve

From the above table no. 1 it is clear that,

1) Alkaloid are present in extract of Distilled water and ethanol and absent in extracts of Petroleum ether, chloroform, acetone, benzene extracts.
2) Favonoids are present in extract of ethyl alcohol and absent in extracts of Petroleum ether, chloroform, benzene, acetone and Distilled water.
3) Anthoquinones are present in extract of ethanol, Petroleum ether, chloroform , acetone , benzene and absent in the extract of Distilled water
4) Coumarin are present in extract of ethyl alcohol and absent in extracts of Petroleum ether, chloroform, benzene, acetone and Distilled water.
5) Proteins, Saponins, Steroids, Tannins, Terpenoid, Emodins, Carbohydrate, Cardiac glycosides, Phenol, Phlobatanins, Anthocynins and Leucoanthocynins are absent in all extracts of ethanol, Petroleum ether, chloroform, acetone, benzene, Distilled water.

From the above table no. 2 it is clear that,

1) Alkaloid are present in extract of Petroleum ether, benzene, Distilled water and ethanol and absent in extracts of chloroform, acetone.
2) Flavonoids are present in extract of Distilled water, Petroleum ether, ethyl alcohol and absent in the extracts of chloroform, benzene, acetone.
3) Steroids are present in extract of Distilled water, Petroleum ether, chloroform, benzene and absent in the extract of ethanol, acetone.

4) Coumarin are present in extract of ethyl alcohol, Petroleum ether, chloroform, benzene, acetone and absent in extract of Distilled water.
5) Proteins, Saponins, Anthoquinones, Tannins, Terpenoid, Emodins, Carbohydrate, Cardiac glycosides, Flavonoids, Phenol, Phlobatanins, Anthocynins and Leucoanthocynins are absent in all extracts of ethanol, Petroleum ether, chloroform, acetone, benzene and Distilled water.

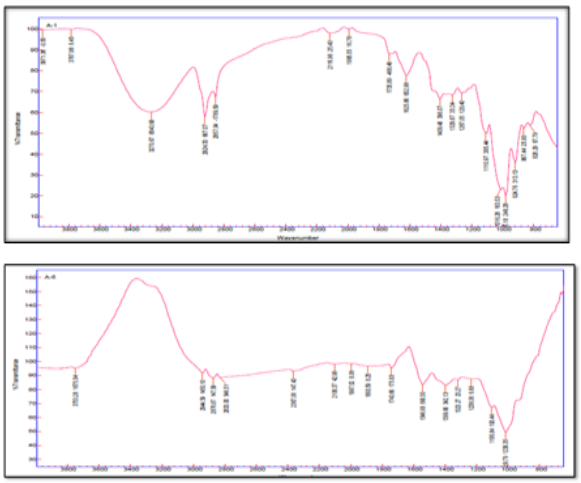


Figure 1:-FT-IR spectrum of *Urginea indica* (Roxb.) Kunth.

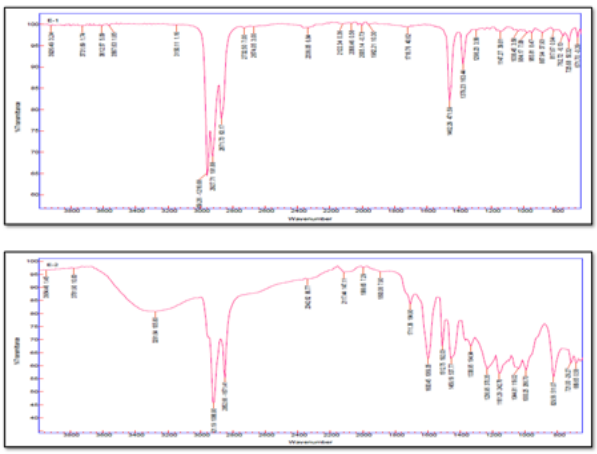


Figure 2:-FT-IR spectrum of *Cyclea peltata* Arn.ex Wight.

IR interpretation

From fig.1,

Ethanol extract (A1) of *Urginea indica* exhibited a characteristic at 3270 cm^{-1} for O–H Stretching, 2924 cm^{-1} for C – H Stretching (alkanes) , at 2857 cm^{-1} for C–H Stretching, at 1736 cm^{-} for C=O stretch, at 1409 cm^{-} for C-C stretch (aromatic) ,at 996 cm^{-1} for Stretching C-Cl (Alkyl Halide) Distilled Water extract(A6) of *Urginea indica* exhibited a characteristic at 3753 cm^{-1} for O-H stretch (carboxylic acid), 2944 cm^{-1} for C – H Stretching (alkanes) , at 2876 cm^{-1} for C – H Stretching, at 1743 cm^{-} for C=O stretch, at 1546 cm^{-} for C-C stretch (aromatic) ,at 1079 cm^{-1} for N-H (primary/secondary amines).

From fig.2,

Ethanol extract(E1) of *Cyclea peltata* exhibited a characteristic at 2959 cm^{-1} for C – H Stretching ,O-H stretch (carboxylic acid), 2927 cm^{-1} for C – H Stretching (alkanes), at 2871 cm^{-1} for C – H Stretching, at 1462 cm^{-} for C-C stretch (aromatic), at 762 cm^{-1} for N-H (primary/secondary amines), at 725 cm^{-} Stretching C-Cl (Alkyl Halide) Acetone extract (E2) of *Cyclea peltata* exhibited a characteristic at 2921 cm^{-1} for C–H Stretching (alkanes), 2852 cm^{-1} for O-H stretch (carboxylic acid), at 1711 cm^{-} for C=O stretch ,at 1600 cm^{-} for C-C stretch (aromatic), at 1512 cm^{-} for N-O stretch, at 1238 cm^{-1} for C-N , at 689 cm^{-} Stretching C-Br.

Table:3 :-Antimicrobial Sensitivity Test Against Some Microbial Pathogen Of *Urginea indica* (Roxb.) Kunth

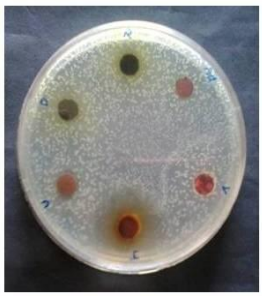
Sr. No.	Plant Extract	*E. coli*	*Pseudomonas fluroscene*	*Salmonella typhi*	*Bacillus subtilis*	*Klebsiella pneumoniae*	*Staphylococcus Aureus*	*Strepto cocus*	Yeast candida	*Aspergillus niger*
1	Ab.Alcohol	++	_	_	+ +	+	_	_	++	_
2	Petroleum Ether	+++	–	–	+++	–	–	–	–	–
3	Acetone	-	_	_	_	_	_	_	_	_
4	Benzene	-	++	++	++	_	_	_	_	_
5	Chloroform	+++	_	_	_	++	_	_	_	_
6	Distilled Water	+	–	–	+++	–	++	+	–	–

N.B. ─ Inactive (Resistance) , + Active (Zone of Inhibition)

Table: 4:-Antimicrobial Sensitivity Test Against Some Microbial Pathogen Of *Cyclea peltata* Arn. ex Wight

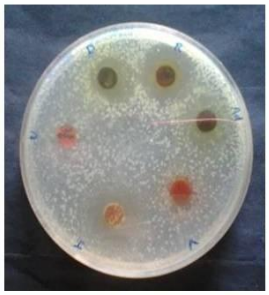
Sr. No.	Plant Extract	*E. coli*	*Pseudomonas fluroscene*	*Salmonella typhi*	*Bacillus Subtilis*	*Klebsiella pneumoniae*	*Staphylococcus Aureus*	*Strepto cocus*	Yeast candida	*Aspergillus niger*
1	Ab.alcohol	+	-	++	+++	-	-	-	-	-
2	Petrolium Ether	+	–	–	–	–	+++	–	–	+
3	Acetone	-	-	-	-	++	-	-	-	-
4	Benzene	+++	+	-	++	-	-	-	-	-
5	Chloroform	+++	-	-	-	-	-	-	-	-
6	Distilled Water	++	-	-	-	-	-	-	-	-

N.B. ─ Inactive (Resistance) , + Active (Zone of Inhibition)

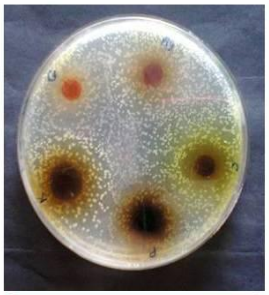


Staphylococcus aureus inhibition of different solvent extract of Cyclea peltata Arn. ex Wight.

E.coli inhibition of different solvent extract of Cyclea peltata Arn. ex Wight.



B.subtilis inhibition of different solvent extract of Urginea indica (Roxb.) Kunth.



E.coli inhibition of different solvent extract of Urginea indica (Roxb.) Kunth.

From table 3 it is clear that *Urginea indica*, the ethanol extract showed activity against E. coli, Bacillus subtilis, Klebsiella pneumoniae, Yeast candida and inactive against Pseudomonas fluroscene, Salmonella typhi, Staphylococcus aureus, Strepto Cocus, Aspergillus niger. The petroleum ether extract showed activity against E. coli, Bacillus subtilis and inactive against all remaining pathogens. The benzene extract showed activity against Pseudomonas fluroscene, Salmonella typhi and Bacillus subtilis.The Chloroform extract showed activity against *E. coli, Klebsiella pneumonia.* The distilled water extract showed activity against E. coli, Bacillus subtilis, Staphylococcus aureus, Streptococus.

From table 4 it is clear that *Cyclea peltata* the ehtanol extract showed activity against E. coli, Salmonella typhi, Bacillus Subtilis. The petroleum ether showed activity against E. coli, Staphylococcus aureus, Aspergillus niger. The benzene extract showed activity against E. coli, Pseudomonas fluroscene, Bacillus Subtilis.

The Chloroform extract showed activity against E. coli. The distilled water extract showed activity against E. coli. The acetone extract showed activity against Klebsiella pneumonia.

Our results were clearly revealed that the plant contained different bioactive compounds such as alkaloids, anthoquinones, flavonoids, steroids are connected with defense mechanism against many microorganisms.

CONCLUSION

Our results were clearly revealed that the plant contained different bioactive compounds such as of Alkaloids, Anthoquinones, Coumarins, Steriods and Flavonoids. These compounds were rich in the extracts of *Urginea indica* (Liliaceae) and *Cyclea peltata* (Menispermaceae) are connected with defense mechanism against many microorganisms. These plants have antimicrobial activity against some gram positive and gram negative bacteria such as, *E.coli, Pseudomonas fluroscene, Salmonella typhi Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, Streptococus, Yeast candida, Aspergillus niger.* Ethanolic extract showed good antimicrobial activity against tested bacterial strains such as *Escherichia coli, Staphylococcus aureus, and Bacillus subtilis Klebsiella pneumoniae, Yeast candida, Salmonella typhi*. Thus these plants can be utilize as an alternative source of useful drug.

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