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Analysis of phytochemical status and antibacterial activities on *Alysicarpus Bubleurifolius* – a valuable medicinal herb

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Abstract

Analysis of Phytochemical status and antibacterial activities work was carried out in *Alysicarpus bubleurifolius* -A valuable medicinal herb. This work was carried out in 3 different extracts that extracts are ethanol, ethyl acetate, aqueous. The preliminary Phytochemical revealed the presence of carbohydrates, alkaloids, phytosterols, tannin and phenolic compounds, terpenoids and the absence of saponins, proteins and amino acids, cardiac glycosides, fixed oils and fats, steroids. In GC-MS study was carried out in this plant and it shows the presence of 19 chemical components. The antibacterial activity of this plant against some pathogenic bacteria.

Keywords

Alysicarpus bubleurifolius, Ethanol, Ethyl Acetate, Aqueous Extracts, Phytochemical Status, Antibacterial Activity, GC-MS

1. Introduction

Nature has been a source of medicinal agents since times immemorial. India is endowed with a rich wealth of medicinal plants. Herbs have always been principal forms of medicine in India and presently they are becoming popular through out developing countries, as people strive to stay healthy in face of chromic stress and pollution and to treat illness with medicine that work in concert with body's own defence. India recognises more than 2500 plant species which have medicinal values. However, large flora is waiting for investigation for their medicinal properties (kirtikar and Basu, 1995).

Plants have been known to be a reservoir of secondary metabolides which are being exploited as source of bioactive substance for various pharmacological purposes. The fact that some of these plants have been used traditionally for centuries and modern scientific studies have shown the existence of good correlation between the traditional or folkloric application of some of these plants further strengthens the search for pharmacologically active compounds from plants (Abba *et al.,* 2009 Egharevaba and kunle 2010 : A balaka *et al.,* 2009) .

Antimicrobial resistance is a global problem that has created immense clinical problem in treatment of infectious diseases. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens. In India, antimicrobial resistance has been reported in for the most predominant pathogenic micro organisms including *S. Auceus, Entorcoccus faecalis, mycobacterium tuberculosis* and *P. aeruginosa.*

Gas chromatography–mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. It is applicable to known the drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples.

2. Materials and Methods

Alysicapus bubleurifolius belongs to the family Fabaceae. Locally known as Sweet Alys. Plant materials were collected from

They were processed by shade drying for one week then powered and packed in polythene bags and stored in cold dark room.

2.1. Qualitative Analysis of Phytochemical Contitutents

Acetone and chloroform extracrt of Rauwolfia tetrophylla (L) was subjected to qualitative tests for the identification of various active contituents (Wagner et al .,1984,Harbone, 1973).

2.2. Extraction

The extracts are preard by Soaking method. The collected plant materials were shade dried - 50g plant powder were taken and soak into 250ml of Acetone and chloreform.

2.3. Test for Carbohydrates and Glycosides

A small quantity of extract was dissolved in 4 ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates and glycosides.

Molisch's test:

The filtrate was treated with 2-3 drops f 1% alcoholic a-naphthol and 2 ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junctipn of two liquids shows the presence of carbohydrates.

2.4. Glycosides

Another portion of extract was hydrolyzed with dilute hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to the following tests to detect the presence of glycosides.

Borntragetr's test:

Hydrolysate was treated with chloroform and the cholroform layer was separated. To this equal volume of dilute ammonia solution was added. Ammonia layer acquires pink colour shows the presence of glycosides.

2.5. Detection of Fixed Oils and Fats

Filter paper test Smal quantity of extract was pressed between the filter paper. Appearance of oil stain on the paper indicates the presence of fixed oils.

Saponification test:

Few drops of 0.5N alcoholic potassium hydroxide was added to small quantity of extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap indicates the presence of fixed oils and fats.

2.6. Detection of Proteins and Free Aminoacids

Small quantity of extract was dissolved in few ml of water and then it was subjected to the following tests.

Millon's test: The above prepared extract was treated with Millon's reagent. Red colour formed shows the presence of proteins and free amino acids.

Biuret test: To the above prepared extract equal volume of 5 % sodium hydroxide and 1 % copper sulphate solution were added. Violet colour produced shows the presence of proteins and free amino acids.

Ninhydrine test: The extract was treated with Ninhydrine rreagent. Purple colour produced shows the presence of proteins and free amino acids.

2.7. Detection of Saponins

The extract was diluted with 20 ml of distilled water and it was agitated in a measuring cylinder of 15 minutes. The formation of 1 cm lyer of form shows the preence of saponins.

2.8. Detection of Tannins and Phenolic Compounds

Small quantity of the extract was taken in water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.

1 5 % Ferric chloride solution
2 1 % solution of gelatin containing
3 10 % sodium chloride
4 10 % lead acetate solution
5 Above findings shows the presence of phenolic compounds and tannins.

2.9. Detection of Phytosterols

Small quantity of extract was dissolved in 5 ml of chloroform. Then this chloroform solution was subjected to the following tests to detect the presence of phytosterols.

Salkowski test:

To 1 ml of above prepared chlorlform solution few drops of concentrated sulphuric acid was added. Brown colour produced shows the presence of phytosterols.

2.10. Detection of Alkaloids

Small quantity of extract was separately treated with few drops of dilute hydrochloric acid and filtered. The filterated was used for the following tests.

1 Mayer's reagent
2 Dragendroff's reagent

The following methods are adopted to study the antibacterial activity of the plant extracts.

- Disc Diffusion method

2.10.1. Disc Diffusion Method

The disc diffusion method provides a simple and reliable test in routine clinical microbiology. In order to find out the

effect of a particular substance on a specific bacteria or fungus. This method consists of impregnating small circular disc of standard filter paper with given amount of a chosen concentration of substance.

The discs are placed on plates of culture medium previously spread with bacterial or fungal inoculums to be tested. After incubation the degree of sensitivity is determined by measuring the inhibition zone produced by the diffusion of the antibiotic substances from the discs into the surrounding medium.

2.10.2. Preparation of Discs

Discs usually consisted of absorbent paper impregnated with the compound (plant extract). It is most convenient to use Whatman No.1 filter paper for preparing the discs. Dry discs of 6 mm diameter were prepared from Whatman No.1 filter paper and sterilized in an autoclave. There dry discs were used for the assay.

2.10.3. Procedure

Circular discs of 6 mm diameter were prepared from Whatman No.1 filter paper and sterilized in an autoclave. These paper discs were impregnated with test compounds (plant extract) in the respective solvents for overnight and placed on nutrient agar potatoes dextrose agar plates seeded with test bacteria or fungus. The plates were incubated at 37°C for 24 hrs for bacteria and 48 hrs for fungus. After the incubation period zone of inhibition around each disc was measured and the diameter was recorded. Here various extracts are used to bacteria. Like methanol, ethanol and chloroform extracts for roots and leaves. Separate discs were prepared by impregnating with only solvent as control. And the values of negative control are deducted from the tested value for analysis. Results were expressed as mean and standard deviation (±) of two separate experiments.

2.11. Microbes Selected for Study

Bacterial species:

1. Escherichia coli
2. Pseudomonus auriginosa
3. Bacillus cereus
4. Staphylococcus aureus

3. Results and Discussion

The present investigation was carried out that the phytochemical analysis and antibacterial activity was studied in various extracts of (ethanol, ethyl acetate, aqueous) *Alysicarous bubleurifolius* against some pathogenic bacteria.

The preliminary phytochemical analysis (Wagner *et al.,* 1984 and Harborne 1973) was done in ethanol, ethyl acetate, aqueous extracts of *Alysicarpus bubleurifolius.* In that work alkaloids present in all extracts by Mayer's test and Dragentroff's test. Carbohydrates present in all extracts by molisch's test and Fehling's test. It is absent in other two extracts. Saponins presence is confirmed only in aqueous extract by Frothing's test. It is absent in other two extracts. Absence of protein and amino acids was confirmed in all extracts by millon's test and biuret's test. Glycosides is absesnt in all extracts. Tannin, phenolic compounds and phytosterols present in all 3 extracts. Fixed oils and steroids are absent in all extracts. Terpenoids is present only ethanol and ethyl acetate extracts. (Table-1)

Table 1. *Phytochemical studies of Alysicarpus bubleurifolius plant on various extracts*

SI.No.	Experiment	Ethanol	Ethyl acetate	Aqeuous
1	Alkaloids	+	+	+
	Mayer's test	+	+	+
	Dragendroff's test	+	+	+
2	Carbohydrates	+	+	+
	Molisch's test	+	+	+
	Fehling's test	+	+	+
3	Saponins Frothing test	_	_	+
4	Proteins and amino acids	_	_	_
	Millon's test	_	_	_
	Biuret test	_	_	_
5	Cardiac glycosides	_	_	_
6	Tannin and phenolic compounds	+	+	+
7	Phytosterols	+	+	+
8	Fixed oils and fat	_	_	_
9	Steroids	_	_	_
10	Terpenoids	+	+	_

The GC-MS analysis was carried out for identification of phytocompounds in IICPT (Indian Institute of Crop Processing and Technology) in Tanjore.

Presence of some phytocompounds was confirmed by GC-MS analysis for this extracts consist of high amount of 3-O-methyl-d-glucose ($C_7H_{14}O_6$, M.W-194), is present when compare with all other compounds.

Such as other compounds are Butanol-3-methyl-formate ($C_6H_{12}O_2$, M.W-116), Lanceol, cis ($C_{15}H_{24}O$, M.W-200), Hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$, M.W-284), Phytol ($C_{20}H_{40}O$, M.W-296), 9,12-Octadienoic acid,ethyl ester ($C_{20}H_{36}O_2$, M.W-308), Ethyl oleate ($C_{20}H_{38}O_2$, M.W-310), Octadecanoic acid, ethyl ester ($C_{20}H_{40}O_2$, M.W-312), (Fig-13). Nonadecane ($C_{19}H_{40}$, M.W-268), 1,2-benzenedicarboxylic acid, diisooctyl ester ($C_{24}H_{38}O_4$, M.W-390), 2,6,10,14, 18, 22-Tetracoasahexane, 2,6,10,15, 19, 23-hexamethyl-, (all-E)- ($C_{30}H_{50}$, M.W-410), Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl-, (E,E,E)- ($C_{20}H_{34}O$, M.W-290), Vitamin E ($C_{29}H_{50}O_2$, M.W-430), Ergot-5-en-3-ol, (3a')- ($C_{28}H_{48}O$, M.W-400), Stigmasterol ($C_{29}H_{48}O$, M.W-412), Tau-sitosterol ($C_{29}H_{50}O$, M.W-414), a-Amyrin ($C_{30}H_{50}O$, M.W-426), Lup-20(29)-en-3-one ($C_{30}H_{48}O$, M.W-424), Lupeol ($C_{30}H_{50}O$, M.W-426).

3.1. Antibacterial Activity

The antibacterial activity of ethanolic, ethyl acetate, aqueous extracts of *Alysicarpus bubleurifolius* against some pathogenic bacteria by disc diffusion method.

All the 3 extracts of the plant showed antibacterial activities against tested bacterial species.

Ethanolic Extract:

The ethanolic plant extracts showed notable activity when compare with other two extracts. The *Staphylococcus aureus* inhibited high level when compare with all other organisms. The *Pseudomonus auriginosa* having notable inhibition zone than the *Escherichia coli* and *Bacillus serues*. *Escherichia coli* and *Bacillus serues* having minimum inhibition zone than the all other organisms. The antibacterial activity of the ethanolic extract compared favourably with that of standard antibiotics (streptomycin). (Table-2). The same work was done in Boscia angustifolia by Hassan et al.,, Oxalis *corniculata* by Raghavendra.M.P *et al.,*, *Pongamia pinnata* by Arote.S.R *et al.,*.

Table 2. *Antimicrobial activity of Ethanol and extract of Alysicarpus bubleurifolius against bacteria by Disc diffusion method*

Organism	Diameter of inhibition zone in cm (Mean#)	
	Ethanol	Standard antibiotics*
Staphylococcus aureus	6.8±0.16	2.3
Pseudomonus auriginosa	6.6±0.02	2.2
Escherichia coli	6.5±0.01	2.1
Bacillus cereus	6.4±0.10	2.2

Ethyl acetate extract :

The ethyl acetate extract exhibited less inhibition zone compare with other twon extracts. In this ethyl acetate extract *Pseudomonus auriginosa* showed maximum zone level compare with other organisms. *Staphylococcus aureus* indicated lesser inhibition zone than the other organisms. (Table-3). The same work was done in *Hardwickia binata* by Gunaselvi.G *et al.,*, *Alysicarpus vaginalis* by Rathi.M.A *et al.,*, *Cassia occcidentalis* by Egharevba *et al.,*, *Albizzia lebeck* by Chulet rahul *et al.,*.

Table 3. *Antibacterial activity of Ethyl acetate extract of Alysicarpus bubleurifolius against bacteria by Disc diffusion method*

Organism	Diameter of inhibition zone in cm (Mean#)	
	Ethyl acetate	Standard antibiotics*
Staphylococcus aureus	6.1±0.01	2.3
Pseudomonus auriginosa	6.3±0.0	2.2
Escherichia coli	6.2±0.01	2.1
Bacillus cereus	6.2±0	2.2

Aqueous Extract:

The aqueous extract of *Alysicarpus bubleurifolius* showed moderate activity when compare with all other extracts. In that *Escherichia coli* showed higher inhibition zone than the all other organisms. *Bacillus sereus* and *Staphylococcus sereus* exhibited less inhibition zone than the *Escherichia coli* and *Pseudomonus auriginosa.* (Table-4), (Fig-27). The same work was done in Boscia angustifolia by Hassan et al.,, Oxalis *corniculata* by Raghavendra.M.P *et al.,*, *Pongamia pinnata* by Arote.S.R *et al.,*.

Table 4. *Antibacterial activity of Aqueous extract of Alysicarpus bubleurifolius against bacteria by Disc diffusion method*

Organism	Diameter of inhibition zone in cm (Mean#)	
	Aqueous	Standard antibiotics*
Staphylococcus aureus	6.1±0	
Pseudomonus auriginosa	6.3±0.0	
Escherichia coli	6.7±0.03	
Bacillus cereus	6.1±0.0	

4. Conclusion

The present investigation was concluded that the phytochemical analysis (qualitative and quantitative method) and antibacterial activities were carried out in *Alysicarpus bubleurifolius*

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