RESEARCH PAPER | Biotechnology | Volume : 5 | Issue : 3 | March 2015 | ISSN - 2249-555X

Phytochemical Analysis and Antimicrobial Studies for the Different Metabolites Produced by Wormwood Plant (Artemisia Absinthium)

KEYWORDS: Artemisia absinthium, Secondary metabolites, antimicrobial assay

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ABSTRACT *In India, the traditional knowledge of medicine, The Ayurveda, evolved more than 5,000 years ago in the Himalayas. The plants are the major sources of currants in ancient world. Now in 21st century also, people are more keen and interested in the traditional medication as these are very safe and no side effect. The one part of the technology was to use the whole plant or a part of a plant to cure diseases but now it is the time to extract and exploit different bioactive compounds from those plant that have many therapeutically activities. In this present study, the major plant secondary metabolites present in Artemisia absinthium were investigated. Ethanolic extract was obtained and qualitative screening identified the presence of alkaloid, flavonoid, carbohydrate, tannin, quinines, protein, phenol and terpenoid. Their antimicrobial assay against different pathogenic microorganisms like gram negative (Pseudomonas syringae, Enterobacter aerogenes and E.coli) and gram positive (Bacillus megaterium, Micrococcus luteus and Staphylococcus aureus) bacteria. Highest zone of inhibition 88mm was obtained against E.coli and less zone of inhibition 11mm and 12mm were shown by Staphylococcus aureus and Pseudomonas syringae respectively.*

INTRODUCTION

Artemisia absinthium is a herbaceous perennial plant, with a hard, woody rhizome. The stems are straight, growing to 0.8-1.2 m (rarely 1.5 m) tall, grooved, branched, and silvery-green. The leaves are spirally arranged, greenish-grey above and white below, covered with silky silvery-white hairs, and bearing minute oil-producing glands; the basal leaves are up to 25 cm long, bipinnate to tripinnate with long petioles, with the cauline leaves (those on the stem) smaller, 5-10 cm long, less divided, and with short petioles; the uppermost leaves can be both simple and sessile (without a petiole). The word "Absinthium" comes from Ancient Greek 'apsinthion' possibly meaning "unenjoyable", and probably referring to the bitter nature of the derived beverage, though this is likely folk etymology. The word "wormwood" comes from Middle English "wormwood" or wermode". The form "wormwood" is influenced by the traditional use as a cure for intestinal worms. The leaves and flowering shoots are anthelmintic, anti-inflammatory, antiseptic, antispasmodic, antitumor, carminative, cholagogue, Emmenagogue, febrifuge, hypnotic, stimulant, stomachic, tonic and vermifuge.

Wormwood is perhaps best known because of the use of its oil to prepare certain alcoholic beverages, most notably vermouth and absinthe. As a traditional medicine, wormwood was used by herbalists as a bitter to improve digestion, to fight worm infestations, and to stimulate menstruation. It was also regarded as a useful remedy for liver and gallbladder problems. Some of the pharmacological applications are mentioned below: Anthelmintic; Antiseptic; Antispasmodic; Carminative; Cholagogue; Emmenagogue; Febrifuge; Homeopathy; Hypnotic; Stimulant; Stomachic; Tonic; Vermifuge. Along with the above-mentioned applications, it is also showing antiparasitic properties due to its α-santonin content, which is recognized as a medicine for parasitic diseases. The plant is poisonous if used in large quantities. The plant contains thujone. In small quantities this acts as a brain stimulant but is toxic in excess.

MATERIAL AND METHODOLOGY

Sample collection

Plant of *Artemisia absinthium* was collected from a nearby Military nursery in Secunderabad, Telengana State.

Sample processing for crude extract

The healthy leaves and stem parts were picked out washed with distill water to remove any dust or dirt particles. Then leaves were shade dried for 7-8 days. After drying, they were grinded to a fine powder and sieved through a muslin cloth to get the finest powder. The powder was then stored in a air tight zipper polythene bag.

Crude extract preparation

The crude extract was extracted from the *Artemisia absinthium* by the process of Soxhelation, in which 10 gram of sieved powder was extracted with the 100 ml. of organic solvent such as 70 % Ethanol, using the Soxhlet apparatus(Borosil®)for about 4-5 hrs. The ethanolic extract of *Artemisia absinthium* thus obtained was collected through outlet and kept opened overnight for vaporization of ethanol. The extract now obtained was concentrated which was deep green in colour.

Qualitative Screening for secondary metabolites

The extracts were further screened qualitatively for the presence of different secondary metabolites by different methods.

Alkaloids were determined by Mayer's test (**Evans, 1997**), Wagner's test (**Wagner, 1993**) and Dragendorff's test (**Waldi, 1965**) ; Tannins by Ferric chloride test (**Mace, 1963**) ; Phenols by Lead acetate test ; Carbohydrates (**Ramakrishnan *et al.*, 1994**) by Fehling, Barfoed and Benedict test ; Glycosides by **Legals test ;** Proteins **(Fisher, 1968; Ruthmann, 1970**) by Millon test and Biuret test ; amino acid by Ninhydrin test (**Yasuma and Ichikawa, 1953**) ; Phytosterols (**Finar, 1986**) Liberdmann-Burchard's test; Fixed oils **(Kokate,1999) ;** Gum and mucilages **(Whistler and Be Miller, 1993).** Other plant secondary metabolites were

Volume : 5 | Issue : 3 | March 2015 | ISSN - 2249-555X

screened were fats, steroids, anthroquinones, terpenoids, glycosides, quinone, saponin, coumarin and flavonoids.

Antimicrobial assay of crude extract by Agar well diffusion method

The antibacterial activity of the different extracts was determined in accordance with Kirby-Bauer antibiotic testing or disc diffusion antibiotic sensitivity testing (**Mohanty A et al, , 2010).** Mueller Hinton Agar plates were prepared, inoculated separatly by suspension culture of *Bacillus megaterium, Micrococcus luteus, Staphylococcus aureus, Escherichia coli, Enterobacter Aerogenes & Pseudomonas syringae* . the whatman filter paper was punched into small circles and 7-8 punched papers were stacked to form one unit. Then these units are the put into 90µl and 100µl containing the secondary metabolites and they absorb the solution containing the plant secondary metabolite . These are then placed in the Agar plated and kept in the incubator for 24 hours at 37^{O}C. During incubation, the secondary metabolite present in the filter paper discs diffuses into the medium and inhibited the growth of the test organisms and the **Minimal Inhibitory Concentration** of secondary metabolite was also calculated for each organism (**Andrews, J. M. (2001))** by agar dilution method.

The ethanolic extract was then desiccated using a desiccators and the concentrated extract was dissolved in Dimethyl sulphoxide (DMSO). 1mg of desiccated sample was first dissolved in 1 ml of DMSO. Then working solutions were prepared ranging from 10µg/ml to 1000µg/ml with an interval of 10 units. The streptomycin antibiotics was taken as positive control and DMSO solvent was taken as negative control.

RESULT

The ethanolic extract of the *Artemisia* plant was screened qualitatively for different secondary metabolites (Table 1).

Table 1 : Qualitative Estimation of Secondary Metabolite of crude extract.

SERIAL NUMBER	Qualitative secondary metabolites	RESULT
1	ALKALOID	+ve
2	FLAVANOID	+ve
3	CARBOHYDRATES	+ve
4	TANINS	+ve
5	SAPONINS	-ve
6	GLYCOSIDES	-ve
7	QUINONES	+ve
8	ANTHROQUINONE	-ve
9	STEROID	-ve
10	COUMARINS	-ve
11	AMINO ACID	-ve
12	PROTEIN	+ve
13	GUMS	-ve
14	PHENOL	+ve
15	TERPENOID	+ve

The crude ethanolic extract was then analysed for antimicrobial assay against *Bacillus megaterium (figure 1), Micrococcus luteus (figure2), Staphylococcus aureus(figure 3), Escherichia coli (figure 4), Enterobacter Aerogenes (figure 5) & Pseudomonas syringae (figure 6)*

Table 2 : Zone of inhibition of plant extract against different pathogenic microorganism

Name of pathogenic organism	Zone of inhibition of ethanolic extract of Artemisia against different pathogenic microorganisms	
	Volume of sample taken	
	90 µl	100 µl
Bacillus megaterium	14 mm	17 mm
Micrococcus luteus	12 mm	19 mm
Staphylococcus aureus	14 mm	11 mm
Escherichia coli	00 mm	00 mm
Enterobacter Aerogenes	14 mm	15 mm
Pseudomonas syringae	No region	12 mm

Fig. 1: *Bacillus megaterium* **at concentrations 90µl & 100µl.**

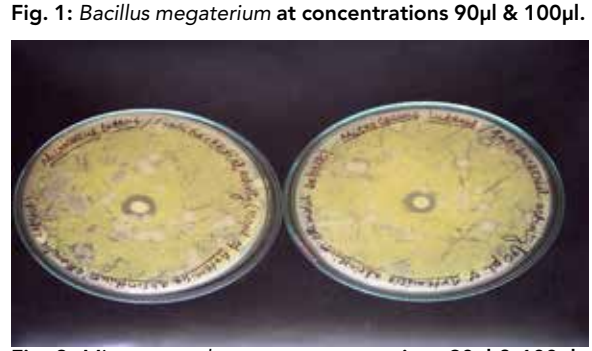

Fig. 2: *Micrococcus luteus* **at concentrations 90µl & 100µl.**

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Fig. 3: *Staphylococcus aureus* **at concentrations 90µl & 100µl.**

Fig 4 : *Escherichia coli* **at concentrations 90µl & 100µl**

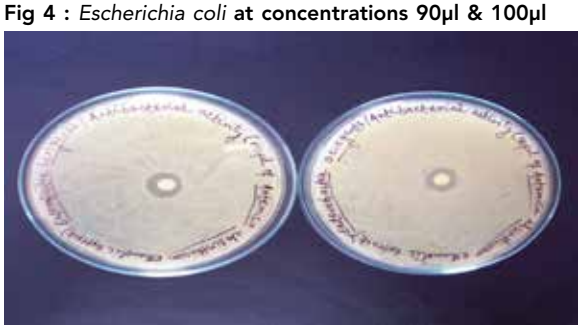

Fig.5: *Enterobacter aerogenes* **at concentrations 90µl & 100µl**

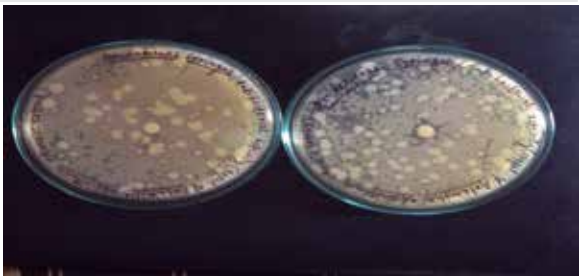

Fig. 6: *Pseudomonas syringae* **at concentrations 90µl & 100µl.**

The minimal inhibitory concentration (MIC) was determined for the ethanolic extract of artemesia against different pathogenic bacteria such as *Bacillus megaterium, Micrococcus luteus, Staphylococcus aureus, Escherichia coli, Enterobacter Aerogenes & Pseudomonas syringae* was carried out.

The minimal concentration of the extract for the bacteria such as *Bacillus megaterium, Micrococcus luteus, Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes & Pseudomonas syringae* were 280µg/ml, 340µg/ml, 220µg/ml, 160µg/ml, 150µg/ml respectively.

DISCUSSION

The ethanolic extract by the hot continous extraction of *Artemisia* contain compound show positive results for Alkaloids, Flavonoids, phenol, terpenoids, quinines and Tannins as the major components. Those extracts also show positive for almost all pathogenic bacteria. Highest activity was seen against *Pseudomonas syringae* and *Enterobacter aerogenes* with concentration of 150µg/ml and 160µg/ml respectively whereas the effectiveness was least against *Micrococcus luteus* with the concentration at 340µg/ml. It was also observed that the extract was more efficiently inhibiting Gram negative bacteria than that of Gram-positive bacteria.

CONCLUSION

Artemisia absinthium can be a potential source of bioactive compound for various pharmaceutical uses as it contains Alkaloids, Flavonoids, phenol, terpenoids, quinines and Tannins. These bioactive metabolites can have antimicrobial, anti-inflammatory, anticancerous antimalarial activities. These plants in general are grown in any soil and agro-climatic zones does not need special cultivation procedures. So these can be used as recycling the waste lands and growing a potential pharmaceutical bioreactor. Recombinant biotechnological applications may increase the productivity of potential biocompounds in these plants.

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