Isolation and Characterization of Active Secondary Metabolites from Some Ethnomedicinal Plants of Rajasthan

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Abstract:

This study aimed to explore the potential medicinal properties of indigenous plants from Rajasthan, India, by isolating and characterizing active secondary metabolites. A total of 3 plant species were collected from various regions of Rajasthan based on their ethnomedicinal significance. The extraction of secondary metabolites was performed using Column Chromatography and Thin Layer Chromatography followed by purification and isolation processes. Characterization of the isolated compounds was conducted employing state-of-the-art analytical methods including chromatography (e.g., HPLC, GC-MS) and spectroscopic techniques (e.g., NMR, IR). The identification of these compounds revealed the presence of [specific compounds] known for their medicinal properties .Furthermore, bioactivity assays were employed to evaluate the pharmacological potential of the isolated compounds. Results indicated significant against .These findings suggest the potential of these ethnomedicinal plants as a source of bioactive compounds for pharmaceutical and therapeutic applications.

Keywords: Ethnomedicina plants, Rajasthan, Secondary metabolites. Isolation, Characterization, Medicinal properties, Phytochemical analysis

Introduction:

Ethnomedicinal plants are the most important source of life saving drugs for the majority of world's population. It is estimated that around 80% of people world-wide rely chiefly on traditional, largely herbal medicines to meet their primary health care needs and have gained renewed interest for various reasons, affordability, low pricing, no side effects, their solutions for dreadly diseases and disorders time tested remedies and preventive approaches. Over the years such medicines have gained upward trend for consumption especially with the development and standardization of herbal medicines (Malik et al., 2008).

Plant derived drugs have a long history in both indigenous and modern system of medicine as herbal remedies or crude drugs, or as purified compounds approved by the Food and Drug Administration and similar regulatory agencies. According to one estimate 20,000 to 35,000 species of these plants are used as medicines, pharmaceuticals, cosmetics and nutraceuticals by different indigenous groups all over the world. In most of these plant species active constituents are exploited in modern medicines and referred to as plants of scientific knowledge.

Increase in the market demands have posed threats to phytoresources due to unscrupulous mode of collections. The old method of cultivation of some of the ethnomedicinal plants is relatively expensive and production of medicinal compounds can be elicited *in vitro.* Due to overexploitation of medicinal plants for medicine and scientific research, many of them are facing danger of extinction; therefore it is imperative to adopt alternative methods for rapid propagation.

In the recent scenario, tissue culture has emerged as a promising technique to obtain genetically pure elite populations under *in vitro* conditions. Plant secondary metabolites are economically important as drugs, fragrances, pigments, food additives and pesticides. Secondary metabolites could be enhanced with the help of bioreactors and has a tremendous potential for the large scale synthesis of therapeutically active compounds in medicinal plants. The major advantages of plant cell culture system over the conventional cultivation of plants are: (1) Important compounds can be produced under controlled conditions independent of climatic conditions; (2) Cultured cells would be microbes free (3) Automated control of cell growth and rational regulation of metabolite processes would reduce labour costs. (4) Organic substances are easily extractable from callus cultures.

According to Alferman et al. (2003), studies have been carried to use plant cell culture for commercial production of plant secondary metabolites. Four commercially viable in vitro secondary metabolite production systems have been created including shikonin (*Lithospermum erythrorhizon*), ginsenosides (*Panax ginseng*), purpurin (*Rubia akane*), and paclitaxel (*Taxus* spec.). Alferman et al. (2003) further studied that the lack of significant application was due to factors including a lack of storage cells for accumulation of these bioactive compounds. In addition, Preil (2005), blamed lack of progress specifically on the high cost of bioreactors, slow growth of the plants, and low yields of active metabolites. Taxol, paclitaxel, is a compound with anticancer properties that was originally extracted from the leaves and bark of plants of the genus *Taxus.* This compound is being successfully produced via cell suspension culture by Phyton Biotech (Germany) in 70,000 L bioreactors (Wink et al., 2005). In most cases, however, callus culture failed to produce significant quantities of medicinal compounds because of the need for compartmentalization of cellular synthetic processes which require differentiation for proper pathway functionality (De Luca and St Pierre, 2000). Immobilization of such suspension cultures was originally conceived as a possible enhancement of the system to allow the productive cells to be maintained while the bioactive compounds were removed with majority of the media (Baldi et al., 2007). Immobilization of *Capsicum* cell cultures allowed for continual production of capsaicin in vitro (Johnson et al., 1990).

The inherent variability in the phytochemical composition of herbal compounds as demonstrated by Bauer and Tittle (1996) can be controlled through the use of culture techniques. *In vitro* culture systems can also be used for specific regulation of medicinal compounds for production of higher value materials.

Methodology

The ethno medicinal plants were collected from the south east area of Rajasthan during the survey conducted. The plants were collected on the basis of their use in curing various health ailments of the indigenous people residing in that particular area. The plants were further identified from the Herbarium in Department of Botany, University of Rajasthan, Jaipur (India). Following ethnomedicinal plants i,e *Argeria speciosa*, *Soymida febrifuga, Peganum harmala, Celastrus paniculata, Gmelina arborea, Oogenia sps, Gymnema sylvestris* depending on the presence of active constituents in them were used for further antimicrobial studies. Following methods were employed for successful extraction of phytochemicals in these ethnomedicinal plants.

EXTRACTION AND ISOLATION OF SECONDARY METABOLITES:-

(i) Extraction:-

Ethanol was selected as initial extraction solvent due to its ability to dissolve a vast variety of compounds in it. Literature also showed that polar extracts also possessed all biological activity.

Plant powder/Callus biomass will be soaked in ethanol in appropriate container and will be regularly shaken for six days at room temperature (28 ± 2°C) in which solvent will be collected and replaced every day (Ghisalberti, 2000). Then it was filtered through Whitman filter paper No. 45. Filtrate was dried under vacuum evaporator at 40°C to yield concentrated dry extract. This extract was stored at 4°C for further investigations.

(ii) Isolation and purification using Column Chromatography and Thin Layer Chromatography (TLC):-

(a) First column: Stationary phase used was silica gel with mesh number of 230-400 and the mobile phase was 20% ethyl acetate/hexane. Dimensions of the column were 3 cm in diameter and 40 cm in height.

(b) Second column: It was prepared to further purify fractions isolated from the first column. Stationary phase used was silica gel with mesh number of 230-400 and the mobile phase was 30% ethyl acetate/hexane. Dimensions of the column were 1.5 cm in diameter and 20 cm in height. Thin Layer Chromatography was performed on fractions isolated from first and second columns.Anisaldehyde-H_2SO_4 spray reagent was used for visualization.

(c) Spectroscopic identification: Infra-red (IR) Spectroscopy or HPLC was conducted on one of the purest fraction isolated from the second column to identify secondary metabolites.

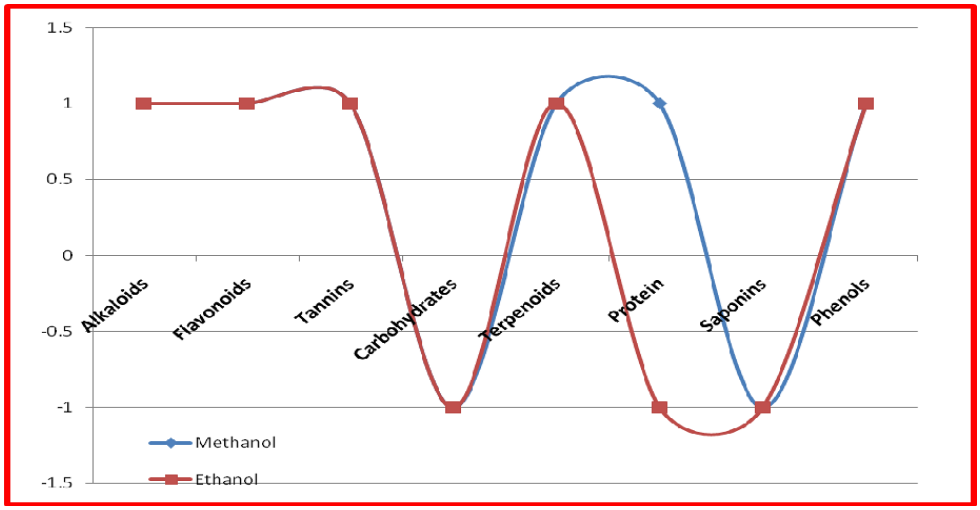

Fig 1 Qualitative phytochemical analysis in Ethnomedicinal plants

IN VITRO ANTIOXIDANT ACTIVITY:-

To screen in vitro antioxidant activity of crude plant extracts and secondary metabolites of these ethnomedicinal plants, the following parameters were assessed spectrophotometrically:-

(i) DPPH Radical Scavenging Activity:-

Radical scavenging activity of leaves extracts of these plants against stable DPPH (1,1-diphenyl-2-picryl hydrazyl radical) was determined by the method of Cuendet et al., (1997). *(ii) Superoxide radical scavenging activity:-*

Superoxide radical scavenging activity of plant extracts and secondary metabolites was measured by the protocol of Beauchamp and Fridovich (1971).

(iii) Hydrogen peroxide scavenging activity:-

This activity was assessed by following the procedure of Ruch et al., (1989).

(iv) Hydroxyl radical scavenging assay:-

This activity was determined by following the protocol of Halliwell and Gutteridge, (1981).

Antimicrobial studies were also carried out in these ethnomedicinal plants

Test microorganisms:

The bacterial strains studied were ***Pseudomonas aeruginosa*** and ***Escherichia coli.*** Microorganisms were maintained at 4 °C on nutrient agar slants. These test organisms were clinical isolates obtained from patients diagnosed for having bacterial infections and procured from SMS Hospital Jaipur.

Antibacterial screening

The filter paper disc method was used for screening the extract for antibacterial activity. Standard size Whatman filter paper disc (6.0 mm diameter) was sterilized in an oven at 140°C for one hour, saturated with plant extracts such as stem and leaf and air dried at room temperature to remove any residual solvent that might interfere with the determination of activity. The discs were then placed on the surface of sterilized nutrient agar medium that had been inoculated with test bacteria (using saline solution) and air dried to remove the surface moisture. The thickness of the agar medium used was kept equal in all the petri plates. Before incubation, the petriplates were placed for one hour in cold room (5°C) to allow the diffusion of the compounds from the disc into the medium. Plates were incubated at 37°C for 20-24 hours .All the experiments were done in three replicates and the activity index was calculated.

$$\text{Activity index (A.I.)} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}$$

Activity index (A.I)

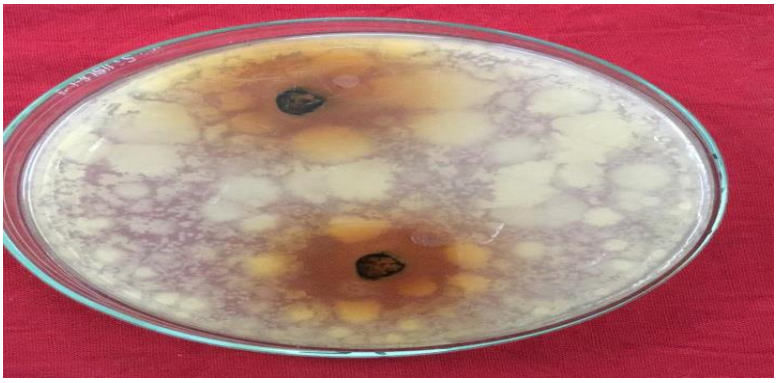

Figure 2.Zone of inhibition in *Celastrus paniculata*

The results of the antimicrobial tests carried out are shown in the following tables.

Table 2: Antibacterial bioassay

Pathogenic bacteria	*Soymida febrifuga*		
	ZOI of Standard	Methanolic Extract	
		Leaves	
		ZOI	AI
Escherichia coli	17.6±0.28		
Staphylococcus aureus	19.07±0.33	30.25±0.29	1.58

Standards: Ciprofloxacin; Activity index = ZOI of test sample/ZOI of standard. Mean±SE, * p< 0.05, ** p<0.01, All columns (ZOI) are compared with standard using Tukey test after ANOVA using ezanova software

Table 3: Antibacterial bioassay

Pathogenic bacteria	*Celastrus paniculata*							
	Standard	Methanolic Extract				Ethanolic Extract			
		Leaves		Stem		Leaves		Stem	
		ZOI	AI	ZOI	AI	ZOI	AI	ZOI	AI
Escherichia coli	17.6±0.28	35.33±1.45	2.00	22.66±0.66	1.28	36.66±0.66	2.08	26.66±0.88	1.51
Pseudomonas aeruginosa	14±0.33	11.00±0.57	0.78	10.33±0.88	0.88	14±0.57	1	12.33±0.88	0.8

Table 4: Antibacterial bioassay

Pathogenic bacteria	*Gmelina arborea*		
	Standard	Methanolic Extract	
		Leaves	
		ZOI	AI
Escherichia coli	17.6±0.28		
Staphylococcus aureus	19.07±0.33	31.00±0.29	1.62

Standards: Ciprofloxacin; Activity index = ZOI of test sample/ZOI of standard. Mean±SE, * p< 0.05, ** p<0.01, All columns (ZOI) are compared with standard using Tukey test after ANOVA using ezanova software

Table 5: Antibacterial bioassay

Pathogenic bacteria	*Gymnema sylvestris*				
	Standard	Methanolic Extract			
		Leaves		Stem	
		ZOI	AI	ZOI	AI
Escherichia coli	17.6±0.28				
Staphylococcus aureus	18.07±0.33	31.66±0.23	1.66	27.66±0.52	1.45

Standards: Ciprofloxacin; Activity index = ZOI of test sample/ZOI of standard. Mean±SE, * p< 0.05, ** p<0.01, All columns (ZOI) are compared with standard using Tukey test after ANOVA using ezanova software

Table 6: Antibacterial bioassay

Pathogenic bacteria	*Peganum harmala*								
	Standard	Methanolic Extract				Ethanolic Extract			
		Leaves		Stem		Leaves		Stem	
		ZOI	AI	ZOI	AI	ZOI	AI	ZOI	AI
Escherichia coli	16±0.28	7±0.57	0.41	8±1.00	0.47	5.66±0.33	0.33	-	-
Pseudomonas aeruginosa	14±0.33	6.33±0.33	0.4	-	-	-	-	-	-

Standards: Ciprofloxacin; Activity index = ZOI of test sample/ZOI of standard. Mean±SE, * p< 0.05, ** p<0.01, All columns (ZOI) are compared with standard using Tukey test after ANOVA using ezanova software

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