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In Vitro studies on antibacterial activity and phytochemical analysis of whole plant extracts of *Stelleria media*

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Abstract

The whole plant extracts of ***Stelleria media*** were examined for the presence of alkaloids and phenolics with simultaneous study on the antimicrobial properties of these extracts. Investigation was carried out using four different concentration (62.5, 125, 250 and 500 mg/ml) of extract to determine the quantitative effectiveness. Preliminary evaluation of both the aqueous and the chloroform fractions showed a broad spectrum of activity (($p<0.05$)) since the extracts inhibit the growth of both gram positive and gram negative bacterial isolates. Clinical isolates used in present investigation were ***E.coli, S.typhi, K. pneumonia, Staph. Aureus, Pseudomonas aeruginosa, Bacillus subtilis.*** The phytochemical analysis revealed the presence of alkaloids, saponins, tannins, flavonoids, cardiac glycosides, anthraquinones and cyanogenetic glycosides in varying concentration. Both the chloroform and aqueous extracts inhibited the growth of the test organisms with ***Salmonella typhi*** and ***E.coli*** showing the highest susceptibility but the pathogens were found to be less sensitive for chloroform extract in comparison to the aqueous extract which could be due to the domination of antimicrobial activity of water soluble polar compounds. This research supports the local use of the plant, ***Stelleria media*** for prophylactic and therapeutic purposes against bacterial infection.

Key words: *Stelleria media*, Chickweed phytochemical analysis, antimicrobial activity, activity index, therapeutic purpose.

Introduction

Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value [1]. Some of them are also used for prophylactic purposes. An increasing interest in herbal remedies has been observed in India from the Vedic Period and many of the herbal remedies have been incorporated into orthodox medicinal plant practice. Diseases that have been managed traditionally using medicinal plant include malaria, epilepsy, infantile convulsion, diarrhea, dysentery, fungal and bacterial infections [2]. Numerous kinds of metabolites have been isolated from various plants and their chemical structure has been elucidated [3-5]. However, the inhibiting activity of such extracts and compounds against microorganisms are poorly investigated [6-8]. *Stelleria media* ("Chickweed") is a plant of Eurasian origin which belongs to the order Caryophyllales which comprises several

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families of herbs and shrubs with simple leaves. It belongs to the family Caryophyllaceae (Pink family). These kinds of plants and herbs are of both nutritional and medicinal importance [9].

Medicinally, chickweed is used as tonic, diuretic, demulcent, expectorant, and mildly laxative. It's often recommended for asthma, bronchitis, or congestion. It's also said to help control obesity and is an ingredient in some herbal weight loss preparations. Externally, chickweed relieves itching and inflammation and is generally soothing and moisturizing. It can be used for any minor skin infections or irritations, and is an ingredient in a number of commercial skin care products. However, the benefits ascribed to chickweed may simply be the result of its high nutritional value, especially the presence of gamma-linolenic acid (GLA). The medicinal effects of this fatty acid read much like the values ascribed to chickweed. GLA is recommended for a variety of skin problems, for hormone imbalances as in PMS, and for arthritis. It clears congestion, controls obesity, reduces inflammation, reduces water retention, acts as tonic for the liver, and reduces the negative effects of alcohol abuse.

Although synthetic and semi synthetic antimicrobial drugs abound in various markets today, there is need for continuous search for new ones to cope with the increased evolution of multiple antimicrobial resistant strains of organisms [10]. The research objectives were to investigate the phytochemical composition of the plant, Stelleria media and its antimicrobial effects on the six test microorganisms.

Materials and methods

Collection and authentication of plant materials

The plants were obtained locally from the cultivated land at and around the Mathura, India. The plant species were identified locally as 'chickweed' and scientifically by experts at Department of Botany, Agra College, Agra as ***Stelleria media***.

Preparation of extracts

To prepare the chloroform extracts, 100 g of each of the ten plant material was collected, dried in the oven at 70^{o}C for 4 h and reduced to powder. It was separately macerated with chloroform, allowed to stand for 72 h and then filtered. The filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. Dried extracts were stored in labeled sterile screw capped bottles at 5°C in the refrigerator, until when required for use. For the aqueous extraction, 50 g of the plant powder was weighed into 50 ml Eylen-Mayer flask and to this was added 400 ml of distilled water. This was heated to boil using hot plate. The mixture was stirred at regular intervals (3-5 min) for eight hours after which it was filtered with No. 1 Whatman filter paper (W and R Balson Ltd, England). The filtrate was then filtered sterilized using a membrane filter of pore size 0.45 cm diameter (Millipore's corp., England). The extracts were concentrated in a hot water bath at 80^{o}C for 5 h during which 0.5 g charcoal was added to decolorize it. Sterile decolorized filtered extract was then refrigerated at 5^{o}C until required for use.

Microorganisms

The species of bacterial organisms were *Staphylococcus aureus, E.coli, K. pneumoniae, Salmonella typhi, Pseudomonas aeruginosa* and *Bacillus subtilis*. They were clinical isolates obtained from **Microbiology Laboratories of DUVASU, Mathura, India**. The cultures of bacteria were maintained on nutrient agar slants at 4°C, re-identified by biochemical tests [11,12] and sub-cultured on to nutrient broth for 24 h prior to testing.

Phytochemical screening

To test for alkaloids, about 0.5 g of the extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath. A few drops of Dragendorff's reagent were used to treat 1 ml of the filterate. Turbidity or precipitation with this reagent was taken as evidence for the presence of alkaloids. Exact 0.5 g of the extract was dissolved

in distilled water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for saponins. Also, to test for presence of tannins, about 0.5 g of the extract was dissolved in distilled water and about 10 ml of bromine water added. Decolourization of bromine water indicated the presence of tannins. Borntrager's test was used for detecting the presence of anthraquinones. In this case 0.5 g of the plant extract was shaken with benzene layer separated and half of its own volume of 10% ammonia solution added. A pink, red or violet coloration in the ammoniacal phase indicated the presence of anthraquinone. The presence of cardiac glycosides was confirmed by Lieberman's test, Salkowski test and Keller-Killani test [13-15]

Antimicrobial susceptibity test

The spreading method of Cruickshank et al. [16] was used. Twenty four hours old cultures of the organisms to be tested were used. A loopful of the cultures was uniformly spread over the surface of a sterile Muller-Hilton agar with a sterile bent rod. The extract was diluted to obtain different concentration of 500, 250, 125 and 62.5 mg/ml using sterile peptone water. Various concentrations of the prepared extracts were used to fill hole bored by 5 mm cork borer in the inoculated agar. The plates were made in triplicate with one for the test organism-tetracycline, standard drug. All plates were incubated at 37^{o}C for 24 h. The diameter of the zones of inhibition in the triplicate plates was measured by calculating the difference between core borer (5 mm) and the diameters of inhibition [17] and their mean designated as ZI. The activity indices, designated as AI, were calculated as the division of zone of inhibition of the extract by that of the standard drug [18].

Tube Dilution Method

The extracts were diluted into different concentrations of 62.5, 125, 250 and 500 mg/ml with sterile peptone water in test tubes. Ethanol and water were used as the control. To each of the dilution was added 0.2 ml broth culture of the test organism. The tubes were incubated at 37°C for 24 h after which turbidity reading was taken using turbidiometer. Extracts added with peptone water served as control.

Total phenolic content determination

A Folin-Ciocalteau method based on Slinkard and Singleton [19] report was applied to determine the total amount of phenolic compounds in different parts of A. paniculata. Working solution samples (20 µl) of each plant part was added to 100 µl of 2N Folin- Ciocalteu reagent as recommended by the above protocol. The mixture was made up to a final volume of 1600 µl using distilled water. Lastly 300 µl of sodium carbonate solution (0.2 mg/ml) was added and incubated at 37°C for 45 min. Absorbance values of the solutions were measured at 760 nm. Total phenolic contents were determined as a gallic acid equivalent (GAE) based on Folin-Ciocalteau calibration curve using gallic acid (ranging from 50 to 1000 mg/ml) as the standard and expressed as mg gallic acid per gram of dry sample.

Determination of pH value

The pH was measured using a pH meter of a glass electrode. The glass electrode was immersed in water for several hours before use. The measurement started about 5 minutes after the equipment was switched on and the detecting unit was rinsed well with water and cleaned with a piece of filter paper. The pH meter was adjusted at one pH value and the temperature compensation dial was rotated to set the temperature of the pH standard solution. The detecting unit was then immersed in the pH standard solution and measurement taken about 2 minutes when the pH meter is set to the pH of the standard solution. The detecting unit was removed from the standard solution, washed well with water and gently blotted with a piece of filter paper to remove water. It was then immersed in the sample solution, and the pH value measured. The temperature of the sample solution and that of the pH standard solution was the same.

Results

The results of the in vitro assays of antibacterial activity and phytochemical analysis of the residues obtained are shown in Tables 1 – 3 and Fig 1. The *in vitro* antibacterial screening of the extracts showed that the crude water and chloroform extracts of the root of *Stelleria media* possess significant (P< 0.05) inhibitory activities against all the tested bacterial isolates with the exception of *P. aeruginosa* at 62.5 mg/ml, respectively, on some of the tested bacteria (Table 1 and 2); with higher inhibitory activity of chloroform extracts compared with aqueous extracts. Preliminary phytochemical screening of the crude extract of *Stelleria media* showed the presence of phenolic compounds (as shown by strong reaction with ferric chloride).

Phytochemistry of the plant extracts

The result of the phytochemical analysis shows that the ten plants are rich in at least one of alkaloids, flavonoids, saponins, anthraquinones, cyanogenetic glycosides, cardiac glycosides and Acidic components. Table shows the phytochemical screening results of aqueous and chloroform extracts of the ten plants used in this study.

Table :1. Phytochemicals in ***Stelleria media*** extracts.

Constituent	AE	CF
Alkaloids	+	++
Glycosides	++	-
Reducing sugar	+	-
Saponins	+	-
Tannins	+	-
Resins	-	++
Flavonoids	-	-
Terpenoids	+	-
Steroids	+	-
Acidic components	++	+
Carbohydrates	+	-
Anthraquinone	+	+
Cardiac glycosides	++	-
Cyanogenetic glycosides	+	+

AE= Aqueous Extract, CF= Chloroform Extract, ++ = Present in appreciable quantity; + = present in low quantity, -= absent

Antimicrobial activity

Both chloroform and crude aqueous forms of the extracts of *Stelleria media* exhibited varying degree of antimicrobial activities against the test organisms. On a general note, aqueous extracts exhibited higher degree of antibacterial activities than the chloroform extracts. At 500 mg/ml, highest zone of inhibition were observed against the *S.typhi* (24 mm) followed by *E.coli* (22mm).Overall data presented in Table.2 revealed that the gram –Ve bacterial strains were found to be more sensitive in comparison to gram +Ve bacteria. Diameter of zone of inhibition decreases with decrease in the concentration of the extract likely became constant at low concentrations. Besides that, aqueous extract had higher antibacterial activities (A.I = 0.9, 0.95 for *Staph.aureus* at 500 and 125 mg/ml respectively). Data presented in table also reveals that extract is more active against the gram –Ve bacteria in comparison to gram +Ve bacteria except for *K.pneumonae*. Activity of extract became decreased at 62.5 mg/ml as highest zone of inhibition was observed for *K.pneumoni* and *B.subtilis* (16mm) while minimum activity index was determined for *P. aeruginosa* (0.43) followed by *B.subtilis* (0.55,0.60) for aqueous extract which is better than chloroform extract (0.52, 0.57 for *Staph.aureus* and *P. aerugenosa* respectively)

Equal or sometimes higher activities were observed at concentration of 500, 250 mg/ml by the aqueous and chloroform extracts than the standard drug, Streptomycin. Hence, the activity index, A.I <1 against E. coli, *Sal. typhi* and *K. pneumoniae*. Consistently high activity indices were observed against the etiology of pneumonia at crude concentration of 250 and 125 mg/ml (Table 2). The high activity indices were enduring with decrease in concentration from 500 to 62.5 mg/ml. Just low reduction in activities were observed as the crude extract concentration were reduced gradually from 500 to 62.5 mg/ml in both, the agar diffusion set up and tube dilution

method (Table 2). The same trend of activity in agar diffusion was equally observed in tube dilution method which was performed for the four important pathogenic isolates i.e. ***E.coli, S.typhi, K. pneumonia, Staph. Aureus,*** Chloroform extract inhibited the growth of the four bacteria with higher turbidity than the aqueous extract. For instance at 500 mg/ml, the turbidity readings were 2.31,2.70,2.10 and 3.13 NTU for crude chloroform extract, while the reading for crude aqueous extracts were 1.50,2.30,1.20 and 3.10 against *Staph. aureus, E. coli, Sal. typhi* and *K. pneumoniae*, respectively. The slight higher potency observed in aqoues extract than chloroform was expected due to antimicrobial activity of polar compounds soluble in water in general. Like the agar diffusion set up, the trend of antimicrobial activity continues until the crude extract concentration of 62.5 mg/ml where both chloroform and aqueous extracts had equal turbidity of 4.56 against E. coli. Meanwhile at this same concentration of 62.5 mg/ml, higher turbidity was observed in chloroform extract tube i.e. 4.82 and 6.60 than in aqueous extract tube i.e. 4.76 and 6.20 against *S. typhi* and *K. pneumonae* in that order.

Total phenolic content

The calculation of total phenolic content of *Stelleria media* extracts was carried out using the standard curve of gallic acid and presented as gallic acid equivalents (GAE) per gram which was determined as 45.5 ±0.25 for the dry aqueous extract of *Stelleria media*.

Discussion

The findings of our research clearly validate the relatively simple *in vitro* system employed in this investigation as a fast and reliable system for *in vitro* screening of plants. The *in vitro* activities of crude plant extracts provide evidence to support the use of such plants [20]. The results obtained from this work revealed that the plants contained bioactive agents which are connected with antimicrobial properties in plants [21]. These agents are alkaloids, saponins, flavonoids, etc. Previous research work revealed that tannins from the barks, roots etc of many plants especially euphorbiaceae are used to treat cells that have gone neoplastic [22]. Presence of flavanoids and phenolics in the extracts also indicate toward the antimicrobial nature of these fractions in clinical use. The pH value of the crude extract was determined and found to tend towards neutrality which indicates that the antimicrobial activity of the plant extract was not affected by the pH. It is obviously interesting to observe the result of high antibacterial effects of both chloroform and aqueous extracts the six potential pathogens of public health importance. *Staph.aureus*, no doubt, is frequently connected to cases of bacteraemia, septicaemia, endocarditis, osteomyelitis, furuncle, etc. It is also frequently involved in both nosocomial and community acquired infections. The successful inhibition of this bacteria and its contemporary aetiology of gastroenteritis (*E. coli*) is a good development, especially when we consider the records of resistance to various conventional antibiotics by them over the years [23-25] These findings are also supported by Adel M. Mahasneh, A. El-Oqlah [26](the petroleum ether crude extracts of the aerial parts of the plants *Stellaria media, Salvia syriaca, Cardaria draba, Euphorbia prostrata Ailon, Rubia tinctoria, Arbutus andrachnel* showed a weak variable degree of antimicrobial activity (2–5 mm inhibition zone) against two gram-positive bacterial species *S. aureus* and *B. cereus*, two gram-negative species for *E. coli* and *S. typhimurium)*. This extract could therefore be of use in management of opportunistic infection in HIV/AIDS involving these two isolates. Similarly, the extract showed appreciable level of potency against the commonest etiology of enteric fever. Records have it that the enteric fever had mortality rate of 10 - 15% in developing countries (Brooks et al., 2004). Both chloroform and aqueous extract could be put into fixed dosage combination therapy for treating the salmonella infection. This extracts is already in use by the traditional medicine practitioners in India, though in either chloroform or aqueous

form. By virtue of high activity indices above unitary value even in crude forms, the extracts have more promising therapeutic advantages than the likes of streptomycin and its aminoglycoside relations when refined to produce antibiotics. Presence of phenolics in aqueous extract indicates the strong antioxidant potential of plant extract.

In conclusion, this finding justifies the traditional use of this plant, *Stelleria media*, for prophylactic and therapeutic purposes. The findings could also be of commercial interest to both pharmaceutical companies and research institutes in the production of new drugs. Further studies on the *in vivo* activity, isolation and structural elucidation of the active component(s), toxicological studies and antitumoural activity of the plant extract are recommended.

Table: 2 Antibacterial properties of *Stelleria media* extract using the agar diffusion technique (mm).

Isolate	Streptomycin (1 mg/ml)	500 mg/ml				250 mg/ml				125 mg/ml				62.5 mg/ml			
		A		B		A		B		A		B		A		B	
		Z.I.	A.I.	Z.I.	A.I.	Z.I.	A.I.	Z.I.	A.I.	Z.I.	A.I.	Z.I.	A.I.	Z.I.	A.I.	Z.I.	A.I.
E.coli	0	22	--	20	--	21	--	19	--	22	--	18	--	13	--	14	--
S.typhi	0	24	--	21	--	22	--	19	--	23	--	21	--	15	--	15	--
K. pneumonia	0	15	--	13	--	16	--	15	--	15	--	13	--	12		16	--
Staph. Aureus	21	19	0.9	19	0.9	15	0.71	15	0.71	20	0.95	19	0.90	11	0.52	11	0.52
Pseud. aeruginosa	21	14	0.67	18	0.86	15	0.71	17	0.81	18	0.86	17	0.81	09	0.43	12	0.57
Bacillus subtilis	20	12	0.6	14	0.7	11	0.55	14	0.7	14	0.70	13	0.65	12	0.6	16	0.8

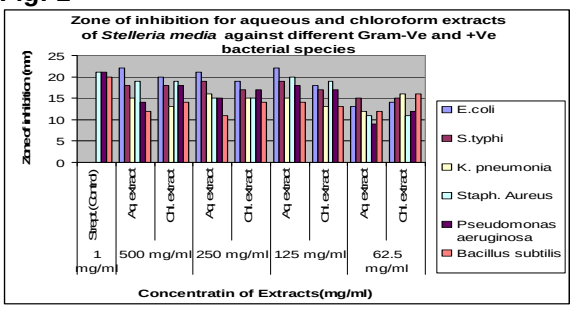
A = aqueous extract, B = Chloroform extract, Z.I = mean zone of inhibition in mm ± SD, A.I = activity Index with respect to Ampicilin.

Table: 3. Antibacterial properties of *Stelleria media* extract using the tube dilution method.

Isolate	500 mg/ml		250 mg/ml		125 mg/ml		62.5 mg/ml	
	A	B	A	B	A	B	A	B
Staph. Aureus	1.50	2.31	2.80	2.81	3.63	3.00	3.90	3.12
E.coli	2.30	2.7	3.32	3.12	4.20	3.30	4.56	4.56
S.typhi	1.20	2.10	2.36	2.80	3.83	3.10	4.32	3.25
K. pneumonia	3.10	3.13	3.83	3.83	4.52	4.12	4.78	4.30
Contol	4.22	4.00	4.12	4.18	4.12	3.95	4.12	4.00

A = aqueous extract, B = Chloroform extract, Values are in NTU

Fig: 1



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