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Review Article

Phytochemical screening, investigations & antimicrobial activity on the various extracts obtained from the aerial parts of *Alternanthera sessilis* (Joyweed) and *Crepidium acuminatum* (Jeevak)

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Total tannin content and Total crude fibre content.

ABSTRACT

The antimicrobial activity of aerial parts of *Alternanthera sessilis* & *Crepidium acuminatum* using petroleum ether, benzene, chloroform, acetone, ethanol and aqueous extracts were tested against Staphylococcus capitis, Staphylococcus mutans, Pseudomonas mirabilis and Bacillus fragillis. The in vitro antibacterial activity was performed by agar disc diffusion method. The zone of inhibition was compared with the standard drug i.e. Penicillin. Petroleum ether, chloroform, acetone and ethanol extracts were effective against the entire four test microorganism used respectively when compared to standard drug penicillin.

The preliminary phytochemical screening of *Alternanthera sessilis* contains alkaloids, terpenoids, flavonoids, tannins, saponins, polyphenols, cardiac glycosides and quinones. *Crepidium acuminatum* indicated the presence of saponins, essential oils, athraquinones, sterols, coumarins, flavonoids, steroids, tannins and glycosides. Quantitatively alkaloids, resins, tannins, crude fibre, saponin, phenolic, flavonoids, carbohydrate and saponins were determined by different methods.

Acetone extract was more effective followed by ethanol extract as antimicrobial agents when compared to other extracts of aerial parts of *Alternanthera sessilis* & *Crepidium acuminatum*. *Alternanthera sessilis* shows antihypertensive remedy, helps to treat hepatitis, bronchitis, asthma, central –stimulating & analgesic activity. *Crepidium acuminatum* shows aphrodisiac, haemostatic, anti-diarrheal, and styptic, anti dysenteric, febrifuge, cooling and tonic activity.

Total Carbohydrate and Saponin contents are calculated by standard curve methods by us ing 80% ethanol and aqueous extract correspondingly. Quantities are found to 112±1.7 (μg Glucose equivalents/ml of extract) in 80% ethanol extract and 184±1.2 (μg saponin equivalents/ml of extract) in aqueous extract. Saponin content has also been measured by double solvent gravimetric method and it is found to be 2±0.48%. Quantity of Total Fat Content, Total Alkaloid Content, Total Resin Content, Total Tannin Content and Total Crude Fibre Content were calculated.

Work is under progress to reveal the chemical nature of the active constituents responsible for the antimicrobial activity & its P'cological activities reported for aerial parts of the plant.

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1. Introduction

1.1. Plant Name: Alternanthera sessilis

Family: Amaranthaceae

Vernacular Names: Marathi: Koprya, Hindi: Gudrisag, English: Sessilie joyweed, Tangle Mat.

1.1.1. Identification

Stems rooting at the nodes, cylindrical and faintly hairy, erect branches.

Leaves are opposite and clustered, simple type, lanceolate to spathulate in shape, obtute apex, entire margin and cuneate base. [1–5]

1.1.2. Inflorescence

White, Axillary spikes, Sessile, Dense,

Seeds/fruits

Fruit: Compressed, small and flat, enclosing the seed

Seeds: Orbicular, dark brown to black

1.1.3. Habit / Habitat

It is a Perennial herb. It typically grows on disturbed parts of wetlands and often observed in a species-rich association with other plants of variety of habitat such as flood-plain wetland, margins of river, streams, canals, ponds, ditches, and damp forest.

1.1.3.1. Uses. The whole plant of *Alternanthera sessilis* is one of three sources of the Ayurvedic drug Lonika, which is used to treat wounds, flatulence, cough, bronchitis and diabetes. This plant is also reckoned as an important ingredient of several compounds of Ayurvedic preparations. The people of Bihar reportedly use the plant to treat night blindness. A poultice of the herb is reportedly useful to promote the healing of boils. The leaves and stems of this herb are used as galactagogue and febrifuge. The fresh and immature shoots and leaves are considered useful for relieving indigestion and are eaten as a cooked vegetable or in soups. To cure inflamed wounds the roots are applied externally. Among the Santhalis and Paharia in eastern Bihar, an extract of the roots, crushed with the bark of Alstonia scholaris (Apocynaceae), is taken with cow's milk for the treatment of spermatorrhoea. [6–10]

1.2. Plant Name: Crepidium acuminatum

Family: Orchidaceae

Vernacular Names: English: The gradually tapering Malaxis, Acuminate malaxis, Jeevak; Sanskrit- Jivaka, Kurcasirsaka, Jiva, Hrisvanga; Hindi- Jivaka; Gujarati- Jivak; Kannada- Jivak; Malayalam- Jivakam; Tamil- Jivakam; Telugu- Jivakamu.

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1.2.1. Habit / Habitat

Around the world it is found in Pakistan, Bhutan and Tibet between 1500-3100 mts elevation. In India found in Central and Eastern Himalaya from Uttarakhand to Assam and Sikkim up to an altitude of 3300 mts in alpine grassland, grassy hill sides, damp gullies and stony slopes. [11,12]

1.2.2. Traditional medicinal uses

1. Decoction prepared from its pseudo bulb is useful in general debility.
2. Intake of powder prepared from its pseudo bulb is useful in seminal weakness.
3. Intake of its pseudobulb powder promotes lactation.

1.2.3. Classical medicinal uses

1. Intake of *Mahamayur Ghrita* processed with *Jivaka* and other herbs is useful in *Rasaraktadi dhatugat vikara, shrotadi indriya vikara* (sensory organ disorders), svarabhransa (Aphesia), asthma, cough, facial paralysis, vaginal disorders, blood disorders and semen related problems.
2. Intake of powder prepared from *Jivaka* and other herbs mixed with an appropriate quantity of honey and crystal sugar is useful in cough and cardiac diseases.
3. *Vacadi Taila* processed with *Jivaka* and other herbs used as anuvasana vasti; is beneficial for Gulma, distention, Vata associated disorders and urinary incontinence.
4. Intake of *Jivaniya Ghrita* processed with Jivaka is useful for the whole body vitiated with gout and Vata associated disorders
5. *Citrakadi Taila* processed with *Jivaka* and other herbs is useful in Vata associated disorders, sciatica, limping, kyphosis, gout and urinary disorders.

2. Objective

Plant material used in the study consisted of aerial parts of *Alternanthera sessilis* (Joyweed leaves) & *Crepidium acuminatum* (Jeevak) is collected from the local area of Himalayas and authenticated by Dr. Anupam Shrivastava, Botany Department, Patanjali Research Institute (PRI), Haridwar, Uttarakhand, India. A voucher specimen is preserved in the Department.

3. Aim & Objectives

1. To study the Phytochemical Profile of selected herbal drugs
2. To perform the Pharmacognostic Investigations of active principles from these Selected plant herbs
3. To study the information of Traditional Medicinal Uses & reported Pharmacological activities of selected herbal drugs.

4. To perform the Antimicrobial Activity on the various Polar and Non-Polar extracts obtained from the aerial parts of *Alternanthera sessilis* (Joyweed) and *Crepidium acuminatum* (Jeevak).

4. Materials and Methods

Preparation of plant extract: The dried aerial parts were coarsely powdered and subjected to successive extraction by Soxhlation. The extraction was done with different solvents in their increasing order of polarity such as petroleum ether, benzene, chloroform, acetone, ethanol and distilled water. Each time the marc was dried and later extracted with other solvents. All the extract were concentrated by rotary vacuum evaporator and evaporated to dryness. 5 mg of the extract was weighed and dissolved in 5ml of DMSO which was labeled as stock 1. From stock 1 further dilution were made so as to get 10, 20, 50, 125, 250, 750 and 1000 μg/ml concentrations by using DMSO as solvent.

4.1. Microorganisms used

All the microbial cultures, used for antimicrobial screening were procured from Microbiology Department, Patanjali Research Institute (PRI), Haridwar, Uttarakhand, India. The bacterial cultures were maintained on Muller Hinton agar slants which were stored at 4^oC.

4.2. Antibacterial activity

4.2.1. Determination of minimum inhibitory concentration (MIC)

The extract were screened for their antibacterial activity in vitro by disc diffusion method [13] using S. capitis, S. mutans, P. mirabilis and B. fragillis as test organism. Agar cultures of the test microorganisms were prepared. Three to five similar colonies were selected and transferred to 5 ml broth with a loop and the broth cultures were incubated for 24 h at 37^oC and suspension was checked to provide approximately 10^{10} colony forming units per ml. 0.1 ml of organism's suspension were spread evenly on the agar plates. For screening, sterile 3 mm diameter disc (Whatman filter paper No. 1) were impregnated with different concentration till saturation, dried and placed in inoculated plates of Muller Hinton agar medium. DMSO solvent was used as negative control. The plates were incubated at 37^oC for

24 h. After incubation for 24 h, the results were recorded by measuring the zones of inhibition surrounding the disc and the lowest concentration of each extract which is showing i nhibition of growth of bacteria was determined as MIC. Penicillin (10μg/ml) was used as standard for bacteria.

4.3. Results and Discussion

4.4. Pharmacological activities of alternanthera sessilis (Joyweed)

1. Wound healing activities: Sunil et al concluded the screening of wound healing activity of chloroform extract leaf of *Alternanthera sessilis* Linn.
2. Anti-oxidant activities: Yadav et al, was aimed to evaluate the antioxidant activity
3. Antipyretic activity: Praveen Singh Nayak et al, reported the antipyretic activity of the ethanol extract of the aerial parts of *Alternanthera sessilis*. (Amaranthaceae) was investigated and claimed the ethno temperature regulatory action in yeast-induced pyrexia in albino rats.
4. Nootropic activity: Rajiv Gupta et al reported the nootropic potential (memory enhancing effects) of the leaves of *Alternanthera sessilis* (Amaranthaceae) on mice.
5. Hepatoprotective Activity: Song-Chow Lin et al reported the hepatoprotective effects of the Taiwanese herb 'Horngtyan-wu' (*Alternanthera sessilis* (L.) DC.) were investigated in three kinds of experimental animal model. [14,15]
6. Hematinic activity: Erna CA et al, The hematinic activity of *Alternanthera sessilis* (L.) R. Br. was investigated by monitoring the change in serum ferritin and hemoglobin levels of mice and rats.
7. Anti- Ulcer Activity: Roy Amit et al were analyzed the invivo antiulcer activity.

4.5. Pharmacological Activities of Crepidium acuminatum (Jeevak)

1. Pseudo bulb is Sweet, aphrodisiac, haemostatic, antidiarrhoeal, styptic, antidysentric, febrifuge, cooling and tonic. It is useful in sterility, vitiated conditions of Pitta and Vata, semen related weakness, internal and external hemorrhages, dysentery, fever, emaciation, burning sensation and general debility.
2. Jivaniya (Vitality promoter)- This medicinal plant is vitality promoter, maintain the balance between three doshas i.e. Vata, Pitta and Kapha. It enhances the energy, body strength, skin glow and other properties of the body.
3. Bramhaniya (Body mass promoter)- This medicinal plant is body mass promoter. It is described within the Bramhaniya varga.
4. Ayushya (Longevity)- This medicinal plant mitigate the disorder of the body and specifically alleviate Tridosaja disorder in the body to increase the longevity and slow down the process of ageing.
5. Antioxidant activity: Pseudo bulb extract of *Crepidium acuminatum* shows antioxidant activity.

6. Antifungal and Antibacterial activity-Extract of *Crepidium acuminatum* shows antifungal and antibacterial activites.
7. The ethanol extract of its pseudo bulb exhibit analgesic and anti-inflammatory activity in experimental animals.

4.6. The antibacterial activity of extract of the aerial parts of Alternanthera sessilis (Joyweed) and Crepidium acuminatum (Jeevak).

The antibacterial activity of extract of the aerial parts of *Alternanthera sessilis* (Joyweed) and *Crepidium acuminatum* (Jeevak). Was capitis, Staphylococcus mutans, Pseudomonas mirabilis and Bacillus fragillis. The results of minimum inhibitory concentration and zone of inhibition are given in Tables 2 and 3.

It is clear from the Tables 1 and 2, Petroleum ether, chloroform, acetone and ethanol extracts were effective against the entire four test microorganism used respectively when compared to standard drug penicillin. The minimum inhibitory concentration [MIC] for S.capitis was 10,75,10,10,10 and 10 mg/ml; MIC for S. mutans was 10,125,10,10,10 and 10 mg/ml; MIC for P.mirabilis was 20, 10,10,10,20 and 10 mg/ml and MIC for B. fragillis was 10,10,10,20,10 and 20 mg/ml for petroleum ether, benzene, chloroform, acetone, ethanol and aqueous extracts respectively suggesting the antibacterial activity of *Alternanthera sessilis* (Joyweed) and *Crepidium acuminatum* (Jeevak). Work is under progress to reveal the chemical nature of the active constituents responsible for the antibacterial activity.

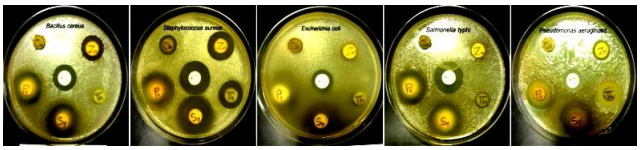

Figure 1: Antibacterial activity.

4.7. Quantification of phytoconstituents

4.7.1. Total resin content

A weighed quantity of sample (5 gm) is refluxed in acetone (3×200 ml) for six h. The extract was filtered and evaporated to dryness. The residue was dissolved in distilled water, warmed and filtered; the residue left was dissolved in solvent ether (2×200 ml). The solvent is evaporated and dried to constant weight at 105°C.

4.7.2. Total tannin content

The powdered sample (2 gm) was extracted with 100 ml distilled water for about 24 h. at room temperature. After 24 h. the mixture was filtered, followed by addition of 5 ml of saturated lead acetate. The addition of lead acetate to the mixture precipitates the tannins as lead tannate. The precipitate was washed with water and dried. Lead tannate obtained was suspended in ethanol, warmed and decomposed by bubbling in H_2S gas. Black precipitate of PbS was removed by filtration and the filtrate concentrated under reduced pressure. The residue obtained was then dissolved in 50 ml water followed by addition of 25 ml of 1% cupric acetate solution. The precipitate thus obtained was washed, dried and incinerated in a muffle furnace keeping all the material in silica crucible and weighed as cupric oxide. The tannin content was calculated by following formula (Ansari, 2010)

% Tannin = (B-A) × 305 × 100/Weight of drug Where, A= Weight of silica crucible

B= Weight of silica crucible and material

4.7.3. Total fat content

Accurately weighed quantity of sample (3 gm) was extracted with anhydrous ether in a Soxhlet apparatus for 6 h. The extract was filtered into a clean dry previously weighed china dish. The extraction flask was rinsed with small quantity of ether, filtered and added to the weighed china dish. The solvent was evaporated and dried to constant weight at 105°C (Ansari, 2004).

4.7.4. Total alkaloid content

Accurately weighed 5 gm of the chloroform extract was mixed with 200 ml of 10% acetic acid in ethanol in 250 ml of a beaker and covered and allowed to stand for 4 h. This was filtered and extract was concentrated on a water bath to one quarter of original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitates were collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloids, which was dried and weighed. [16–20]

Saponin content

Saponin content of the sample was determined by double solvent extraction gravimetric method. 2 gm of the powdered sample was mixed with 50 ml of 20% aqueous ethanol solution. The mixture was heated with periodic agitation in water bath for 90 minutes at 55°C. It was filtered through Whatman filter paper. The residue was extracted with 50 ml of the 20% aqueous ethanol and both extracts were pooled together. The combined extract was reduced to about 40 ml at 90°C and transferred to a separating funnel where 40 ml of diethyl ether was added and shaken vigorously. Separation was done by partition during which the ether layer was discarded and the aqueous layer reserved. Re- extraction by partition was done repeatedly until the aqueous layer become clear in colour. The saponins were extracted with 60 ml of normal butanol.

Table 1: Phytochemical Analysis of the various extract of the aerial parts of *Alternanthera sessilis* (Joyweed) and *Crepidium acuminatum* (Jeevak).

Constituents	Test	Aqueous extract	Ethanolic extract	Benzene extract	Acetone extract
Alkaloids	Hager's Test	-ve	-ve	-ve	-ve
	Mayer's Test	+ve	+ve	+ve	+ve
	Dragendroff's Test	-ve	-ve	-ve	-ve
	Wagner's Test	+ve	+ve	+ve	+ve
Carbohydrates	Molish's Test	+ve	+ve	+ve	+ve
	Fehling's Test	+ve	+ve	+ve	+ve
	Benedict's Test	+ve	+ve	+ve	+ve
Flavanoids	Shinoda test	+ve	+ve	+ve	+ve
	Sodium hydroxide Test	+ve	+ve	+ve	+ve
Tannin and polyphenol	Lead acetate solution	+ve	+ve	+ve	+ve
	5% $FeCl_3$ solution	+ve	+ve	+ve	+ve
	Bromine water test	+ve	+ve	+ve	+ve
	Potassium dichromate test	+ve	+ve	+ve	+ve
Cardiac glycosides	Baljet's test	-ve	-ve	-ve	-ve
	Legal's test	-ve	-ve	-ve	-ve
Anthraquinone glycosides	Borntrager's test	+ve	+ve	+ve	+ve
	Modified Borntrager's test	+ve	+ve	+ve	+ve
Saponin glycoside	Foam test	-ve	-ve	-ve	-ve
Fixed oil	Stain test	-ve	-ve	-ve	-ve
Triterpenoids	Liebermann- Burchard test	+ve	+ve	+ve	+ve
Proteins and Amino acid	Millon's test	+ve	+ve	+ve	+ve
	Biuret test	+ve	+ve	+ve	+ve
	Ninhydrin test	+ve	+ve	+ve	+ve

Table 2: MIC values of different extracts of aerial parts of *Alternanthera sessilis* (Joyweed) and *Crepidium acuminatum* (Jeevak). studied by employing disc diffusion method against Staphylococcus

Microorganism used	MIC with concentration of extract [mg/ml]					
	Petroleum ether	Benzene	Chloroform	Acetone	Ethanol	Water
Staphylococcus capitis	10	75	10	10	100	10
Staphylococcus mutans	10	12	10	10	10	10
Pseudomonas mirabilis	20	10	10	10	20	10
Bacillus fragillis	10	10	10	20	10	20

Table 3: Zone of inhibition values (mm) of different extracts of *Alternanthera sessilis* (Joyweed) and *Crepidium acuminatum* (Jeevak).

Microorganism used	Zone of inhibition (mm) of extracts and standard						
	Petroleum ether	Benzene	Chloroform	Acetone	Ethanol	Water	Penicillin
Staphylococcus capitis	8	8	8	8	8	8	12
Staphylococcus mutans	7	7	7	8	7	7	11
Pseudomonas mirabilis	7	7	8	8	8	7	7
Bacillus fragillis	7	7	8	8	7	7	10

Table 4: Quantification of phytoconstituents of *Alternanthera sessilis* (Joyweed) and *Crepidium acuminatum* (Jeevak).

S.No.	Class of Constituents	Quantity of phyto-constituent
1.	Total Fat Content	1.45 ± 0.13%
2.	Total Alkaloid Content	5±0.52%
3.	Total Resin Content	0.9±0.1 %
4.	Total Tannin Content	9.25±0.21%
5.	Total Crude Fibre Content	5.1±0.69%
6.	Total Saponin Content	2±0.48%
7.	Total Carbohydrate Content	112±1.7 (μg Glucose equivalents/ml of extract) in 80% ethanol extract
8.	Total Saponin Content	184±1.2 (μg saponin equivalents/ml of extract) in aqueous extract

The combined extract were washed twice with 10 ml of 5% aqueous NaCl solution and evaporated to dryness in a pre-weighed evaporating dish. It was dried at 60°C in the oven and reweighed. Percentage saponins were calculated.

% Saponins= (W2 – W1) × 100/ Weight of sample

Where, W_1 = Weight of evaporating dish; W_2 = Weight of dish + sample.

4.7.5. Total carbohydrates content

The total Carbohydrates contents of the plant extract were determined according to the DNS procedure method (Miller, 1959) by some modification that is addition of 15 ml distilled water. Reagents: Potassium sodium tartrate (40% w/v) and Dinitrosalicylic acid (DNS) reagent were prepared, Prepared dilutions for glucose (standard) of 50, 100, 150…., 500 μg/ml.

Sample Preparation: 100 mg. of powder were prepared with 80% ethanol twice (5 ml each time). Collected supernatant and evaporated it. Then added 10 ml of distilled water to dissolve the sugars. 3 ml of solution was taken. To this added 3 ml of 3,5- Dinitrosalicylic acid (DNS reagent) and heated in boiling water bath for 5 min.[21–26]

Preparation of standard: 0-500μg/ml of standard dilutions of glucose was taken and added water to make final volume 3 ml. To this added 3 ml of DNS reagent and heated in boiling water bath for 5 minutes. Added 1 ml of sodium potassium tartrate followed by 15 ml distilled water. Kept for cooling and absorbance was taken at 510 nm.

Blank solutions: 3 ml of distilled water and 3 ml of DNS reagent. Heated in boiling water not Heat bath for 5 minutes. Added 1 ml of sodium potassium tartrate followed by 15 ml distilled water. Kept for cooling and absorbance was taken at 510 nm.

After taking the absorbance of standard dilutions, calibration curve was plotted. Reducing sugar content in drug was calculated by using standard calibration curve.

4.7.6. Crude fibre content

2 gm of the powdered drug was accurately weighed and extracted with petroleum ether. Then 200 ml of 1.25% of sulphuric acid was added to the marc and the whole mixture was boiled for 30 minutes under reflux. Then, the mixture was filtered and residue was washed with boiling water until free from acid. The residue was rinsed back with 200 ml of 1.25% of sodium hydroxide solution. The mixture was boiled for 30 minutes under reflux. The liquid was filtered and residue washed with boiling water until neutral, dried at 110°C to constant weight and then, incinerated to constant weight. The difference between the weight of the dried residue and incinerated residue was calculated with reference to the dried sample (Mukherjee, 2002).

4.7.6.1. Total saponin content. Total saponin determination was done using anisaldehyde reagent. Sample solution was prepared in water. For total saponins estimation 500 μl of sample, 500 μl of 0.5% anisealdehyde reagent, were mixed and kept aside for 10 min. Later, 2 ml of 50% sulphuric acid reagent was added and tubes were mixed. Tubes were then kept in water bath with constant temperature of 60°. After 10 min tubes were cooled and absorbance was taken at 435 nm. The amount of saponins was calculated as saponin equivalent from the calibration curve of standard saponin (100-1000 μg/ml) (Vador et al., 2012).

4.7.6.2. Result. Total Carbohydrate and Saponin contents are calculated by standard curve methods by us ing 80 % ethanol and aqueous extract correspondingly. Results are shown in Table 3. Quantities are found to 112±1.7 (μg Glucose equivalents/ml of extract) in 80% ethanol extract and 184±1.2 (μg saponin equivalents/ml of extract) in aqueous extract. Saponin content has also been measured by double solvent gravimetric method and it is found to be 2±0.48%. Quantity of Total Fat Content, Total Alkaloid Content, Total Resin Content, Total Tannin Content and Total Crude Fibre Content has been represented in percentage in Table 4.

5. Conclusion

Quantification of phytoconstituents of fats, carbohydrates, alkaloids, saponins, flavonoids, phenolics, crude fibres, tannins, and resins were attained. This investigation

confirmed that the plant hold plenteously phytochemicals, which can further be isolated. The above results suggest that acetone extract was more effective followed by ethanol extract as antibacterial agents when compared to other extracts of aerial parts of *Alternanthera sessilis* (Joyweed) and *Crepidium acuminatum* (Jeevak). Quantity of Total Fat Content, Total Alkaloid Content, Total Resin Content, Total Tannin Content and Total Crude Fibre Content has been reported.[27,28]

6. Source of Funding

None.

7. Conflict of Interest

None.

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