

Biological screening of three selected folklore medicinal plants from Pakistan

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Abstract: This study investigates the pharmacological potential of *Adiantum incisum*, *Alternanthera pungens* and *Trichodesma indicum*. Biological activities of plant extracts (aqueous, methanolic and n-hexane extracts of whole plants) were screened by antitumor potato disc assay (10000, 1000, 100, 10 ppm doses), antifungal tube dilution assay (50, 25, 12.5, 6.25mg/ml) and antioxidant DPPH/reducing power assays (250, 200, 150, 100, 50µg/ml). Significant amount of alkaloids (230.83 ± 30.20 mg/g) in *Adiantum incisum* with lowest amount of phenolics in *Alternanthera pungens* (43.45 ± 14.22 µg/mg) were detected. Significant antitumor potential ($p < 0.05$) was revealed by *Trichodesma indicum* n-hexane extract (85% tumor inhibition; $IC_{50} < 10$ ppm). Moderate to significant antifungal activity (50.73%-78.3%) was shown against *Aspergillus niger* by *Adiantum incisum* extracts. Hexane extract of *Trichodesma indicum* revealed significant antifungal activity (98.9% inhibition) against *Mucor* specie. Methanolic extracts of all plants displayed significant DPPH radical scavenging potential (96.72%-60.33%; $IC_{50} < 50$ µg/ml) and Ferric power reducing ability with absorbance values (0.164-0.942) very close to standard ascorbic acid. Present study supports the extensive use of these plants in folk medicine and also promotes elaborative *in-vivo* investigations, isolation of pure therapeutic compounds and formulation of plant-based drugs.

Keywords: *Adiantum incisum*, *Alternanthera pungens*, antitumor potential, phytochemistry.

INTRODUCTION

Man has been informed about the medicinal benefits of plants as early as 5,000 years ago (Pal & Shukla 2003). Reports of WHO (World Health Organization) indicate that approximately 60-80% of people in poor countries rely on old medicinal procedures, mostly plant drugs, for the primary health care (Hassan *et al.*, 2009). 25-50% of medicines are directly or indirectly derived from plants (Dzotam *et al.*, 2016). Many advanced drugs such as aspirin, atropine, ephedrine, digoxin, morphine, quinine, reserpine, and tubocurarine have been formulated due to knowledge obtained by traditional medicinal practices (Rahmatullah *et al.*, 2012). There are reports which indicate that business of medicinal plants around the world shall increase to five trillion US dollars until the year 2050 (Shinwari & Qaiser 2011). Therapeutic properties of plants are due to production of secondary metabolites which are produced in response to different environmental conditions or as defense mechanisms. Different factors, such as climate change, season, temperature, light, salinity, humidity, radiation, heavy metal stress, nutrient deficiencies and pathogen attacks, influence and regulate the quality and quantity of phytochemicals in plants. It has been reported that polyphenol production and accumulation is usually increased as a result of biotic or abiotic stress signals. Cold stress or over wintering stimulates production of soluble sugars and nitrogenous compounds (Ramakrishna

& Ravishankar, 2011). Particular areas are responsible for providing necessary conditions which results in the sustainable production of bio-active agents in medicinal plants (Acharya *et al.*, 2010). Therefore, it is important to consider the area and environment of medicinal plant while characterizing its pharmacological potential. Plant based medicines are gaining worldwide importance because of number of advantages including natural origin, stabilized composition, minimum side effects, target specific activity, bio-degradability, environmentally safe and non-toxic (Sen *et al.*, 2010). Plants have been utilized and possess considerable bright prospects as potent antitumor, anticancer, antimicrobial and antioxidants agents (Sati *et al.*, 2010). Although many plants are utilized as drugs but the active pathways, phyto-constituents and specific targeted activity against particular diseases or organisms are yet to be explored for many medicinal plants. This quest leads towards extensive research for evaluating pharmacological potential of different medicinal plant species. Three different plant species with well-known folk medicinal uses were selected for their pharmacological characterization from an unexplored locality Kund village, Pakistan. *Adiantum incisum* Forssk (Pteridaceae) is a fern and it is used for curing skin diseases, cough, cold, hemicranias, fever, internal burning of body, jaundice and liver problems. It is also reported that this plant possesses anti diabetic activity (Fatima *et al.*, 2012; Haq *et al.*, 2011; Shukla *et al.*, 2011). *Alternanthera pungens* Kunth (Amaranthaceae) is a low creeping perennial herb and it is used in folk medicine for the

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treatment of mouth ulcer, cough, malaria and fever (Konea *et al.*, 2007). *Trichodesma indicum* L. (Boraginaceae) is an annual herb and it is used for curing fever, arthritis, dysentery, diarrhea, eye diseases, skin diseases, snake-bite poisoning and inflammations (Perianayagam, *et al.*, 2011).

Phyto-chemical screening, anti-tumor, anti-fungal and antioxidant assays were performed to analyze the pharmacological significance of these selected plants. To the best of our knowledge, experiments designed in this study for determining the pharmacological importance of the selected plant species are novel and whole plant extracts analyzed in three solvents of different polarity have not been reported elsewhere.

MATERIALS AND METHODS

Collection and identification of plants

Adiantum incisum Forssk, *Alternanthera pungens* Kunth and *Trichodesma indicum* L. were collected from Kund village in July 2013. Kund is a small village located near Union Council i.e. Nara. Nara is the Union Council of Tehsil Kahuta. Tehsil Kahuta is one of the seven tehsils of Rawalpindi District. Plants were identified from Department of Plant Sciences, Quaid-e-Azam University, Islamabad and specimens of the collected plants were saved in herbarium. Voucher Numbers of Plants are: *Adiantum incisum* 2978-ZB, *Alternanthera pungens* 1487-BA and *Trichodesma indicum* 2564-MA.

Extracts preparation

Shade dried whole plants were powdered and extracted with three different solvents of distinct polarity index, water (9.0), methanol (5.1) and *n*-hexane (0.0). Extracts were prepared by soaking plant powder in distilled water, methanol and *n*-hexane respectively for 15 days. Solutions were filtered with single layer of gauze cloth followed by Whatman filter paper no. 41. (8 μ m) Clear extracts obtained were evaporated to dryness in a rotatory evaporator (Sigma-Aldrich) at 30°C $\pm$ 2. Dried extracts were kept in refrigerator at 4°C for further experimental procedures.

Phytochemical screening (Qualitative and quantitative)

Different biochemical tests were employed as reported by Gupta *et al.* (2013) with certain modifications.

- **Alkaloids:** 2-3 drops of Dragendorff's reagent were added in 1ml of each plant extract and formation of orange-brown color shows the presence of alkaloids.
- **Anthraquinones:** Few drops of 2% HCl were added in samples and red color precipitate indicates the presence of anthraquinones.
- **Cardiac Glycosides:** 2ml of acetic acid and few drops of FeCl₃. 1ml H₂SO₄ were added in 1ml of samples and formation of brown ring at interface confirmed presence of cardiac glycosides.

- **Coumarins:** 10% NaOH was used and yellow color formation indicated coumarins.
- **Flavonoids:** 1ml of NaOH (2N) was added and yellow color formation indicates the presence of flavonoids.
- **Glycosides:** Chloroform and Conc. H₂SO₄ (both of them 2ml) were added in 1ml of each plant extract. Glycine portion in glycosides is indicated in plant extracts due to the presence of steroidal ring which is of reddish-brown color.
- **Phenols:** 1ml of 5% FeCl₃ was added in samples and blue or green color formation indicates phenols.
- **Phlobatannins:** Few drops of ammonia were added in samples and appearance of pink color precipitate indicates phlobatannins.
- **Quinones:** 1ml H₂SO₄ was added and red color formation shows the presence of quinones.
- **Saponins:** 20ml of distilled water was added in samples and diluted solution was shaken vigorously. Formation of foam layer indicates saponins.
- **Steroids:** 1ml of chloroform and few drops of H₂SO₄ were added and appearance of brown ring or bluish brown ring indicates the presence of steroids.
- **Tannins:** 2ml of 5% FeCl₃ was added in samples and formation of dark blue or greenish black color indicates tannins.
- **Terpenoids:** 2ml of chloroform and 1ml of concentrated H₂SO₄ was added in samples and presence of terpenoids was confirmed by formation of brown ring at the interface.

Quantitative analysis

Total alkaloids, flavonoids, saponins, phenolics and tannins content were determined in selected plant species according to the methods reported (Maurya *et al.* (2010); Mbaebie *et al.* (2012); Tamilselvi *et al.* (2012); Ibrar *et al.* (2017).

Estimation of total alkaloids

2.5 grams of each plant powder was added to 100ml of 10% CH₃COOH in C₂H₅OH. After 4 hours, it was filtered and filtrate was reduced to 1/4th. NH₄OH was added drop-wise until precipitation. Precipitates were washed with dil. NH₄OH and filtered. The collected residue was dried and weighed. Total alkaloid contents were estimated using the following formula.

Percentage of alkaloids = (final weight of sample / initial weight of plant powder) x 100

Estimation of total flavonoids

2.5 grams of each plant powder was added to 25ml of 80% aqueous methanol. Solution was filtered and it was evaporated to dryness and weighed. Flavonoids were estimated using the following formula:

Percentage of flavonoids = (final weight of sample / initial weight of plant powder) x 100

Estimation of total saponins

2.5grams of each plant powder was added in 25ml of 20% aqueous ethanol. Mixture was reduced at 55°C and filtered. Concentrated solution was taken into a separating funnel. Then 5ml of diethyl ether was added into the funnel and now whole solution was shaken vigorously. Two separate layers were formed, aqueous layer was collected and ether layer was discarded. 10ml of butanol was added and the whole mixture was washed with 5ml of 5% aqueous NaCl. The solution was heated and oven dried at 40°C to a constant weight. Total saponins content was estimated using the following formula:

Percentage of saponins = (final weight of sample / initial weight of plant powder) x 100

Estimation of total phenolics

Different concentrations (10-100µg/ml) of gallic acid were prepared in methanol. 0.5ml from different concentrations of gallic acid was taken and 2.5ml of 10 fold diluted Folin's reagent and 2ml of 7.5% Na₂CO₃ was added to these gallic acid concentrations. Solutions were incubated for 30 minutes and absorbance read at 760nm. 0.5ml of sample was treated with similar procedure as gallic acid. Total phenolic content was determined by putting the value of absorbance in standard curve equation, where y indicates the absorbance of sample at 760nm and x indicates the total phenolic content.

Estimation of total Tannins

0.1ml from different concentrations of gallic acid was taken and 0.5ml of conc. Folin's reagent and 1ml of 35% Na₂CO₃ was added to these gallic acid concentrations. Solutions were allowed to incubate for 30 minutes and absorbance was read at 725nm with a UV-Vis Spectrophotometer. 0.1ml of sample treated with similar procedure. Total tannins content was determined by putting the value of absorbance in standard curve equation where y is the absorbance of sample at 725nm and x is the total tannins content.

Anti-tumor assay

Potato disc method was used according to the described protocol (Karakas *et al.*, 2012) *Agrobacterium tumefaciens* (At10) was cultured on Luria Broth media at 28°C for 48 h. Red skinned potatoes were sterilized with mercuric chloride solution followed by washing thrice with distilled water. Potato discs (8 mm) were prepared with sterilized borer which were placed on 1.5% agar plates. DMSO was used as negative control and Vincristine (0.25mg/ml) (SIGMA-ALDRICH purity 95.0-105.0%) was used as positive control. 50µl of inoculum containing bacterial culture, autoclaved distilled water and sample (10000, 1000, 100 and 10ppm) was poured on potato discs. Petri plates were wrapped with parafilm and placed in incubator at 28°C±2°C for 21 days. After 21 days, Lugol's solution (10% KI, 5% iodine) was used for staining. Creamy to yellow tumors formed on discs were

counted with the aid of 3X magnifying glass and their percentage inhibition was calculated by the following formula. Percentage inhibition = 100 - (average no. of tumors on test sample / average no. of tumors on negative control) × 100

Antifungal assay

Agar tube dilution method (Bibi *et al.*, 2011) was used for detecting antifungal potential. SDA (Sabouraud dextrose agar) (3ml) was poured in screw capped test tubes and autoclaved at 121°C at 15 psi pressure for 30 min. Tubes were allowed to cool at 50°C and 150 µl of plant extracts with different concentration were poured in tubes. Positive and negative control was also prepared by inoculating 150µl of Fluconazole (6.25 mg/ml) (SIGMA-ALDRICH purity >98%) and DMSO in SDA respectively. Tubes were shaken and allowed to solidify in slanting position at room temperature (25°C±5). After solidification of SDA in slanting position, approximately 4mm piece of each freshly grown fungal strain (*Aspergillus flavus*, *Aspergillus niger* and *Mucor* spp.) was inoculated in the center of SDA slants. Tubes were incubated at 28°C for 7 days. After seven days of incubation, results were calculated by measuring linear growth of the control and test samples while growth inhibition was calculated by the following formula. Percentage inhibition of fungal growth = 100 - [linear growth in samples (mm) / linear growth in control (mm)] × 100

Antioxidant assays

DPPH free radical scavenging assay and reducing power assay were performed according to reported methods (Sahu *et al.* 2013; Safdar *et al.*, 2016). Different concentrations (250, 200, 150, 100 and 50 µg/ml) of plant extracts were used to determine their antioxidant activity. Ascorbic acid (SIGMA-ALDRICH) was used as positive control.

DPPH free radical scavenging assay

1.5ml of samples were mixed with 1.5ml of (0.1mM) DPPH solution. Ascorbic acid served as standard. Negative control was prepared by mixing 1.5ml of methanol and 1.5ml (0.1mM) DPPH solution. All solutions were incubated for 30 minutes at room temperature in dark and absorbance was read at 517nm. Percentage inhibition was calculated using the formula:

Inhibition percentage = (Ac-As/Ac) x100

Where Ac = absorbance of control

As = absorbance of sample

Reducing power assay

0.50ml of respective plant extract was mixed with 0.50ml of phosphate buffer and 0.50ml potassium ferricyanide. Solution was evaporated at 50°C for 20 minutes. After cooling, 0.50ml of 10% trichloro acetic acid was added in

it. The solution was centrifuged at 3000 rpm for 10 minutes and 1.5ml of distilled water and 0.1ml of 0.1% FeCl_3 was added to supernatant. The solution was then incubated for 10 minutes and absorbance was read at 700nm. High value of absorbance indicates high reducing power.

STATISTICAL ANALYSIS

All the experiments were carried out in triplicates. The values were expressed as the means $\pm$ standard deviation (S.D.) of three observations in each group. One-way and two-way ANOVA tests were performed using data analysis tools in excel for calculating P value. P value less than or equal to 0.05 was considered to be significant.

RESULTS

Phytochemical screening

Alkaloids, flavonoids, tannins, terpenoids, glycosides, phenols and saponins are the well-known phyto-constituents which were present in all three plants. Anthraquinones were absent in all the extracts of plant samples whereas phlobatannins were present only in methanol and aqueous extracts of *A. incisum* and *A. pungens* respectively (table 1a). Qualitative phytochemical analysis for *Adiantum incisum* extracts showed the presence of important phytochemicals like alkaloids, glycosides, saponins, tannins and terpenoids.

Phytochemical quantitative analysis (table 1 b) revealed increased quantity of alkaloid contents (mg/g) in different plants in the following order: *Adiantum incisum* (230.83 ± 30.20) > *Alternanthera pungens* (219.66 ± 16.11) > *Trichodesma indicum* (208.13 ± 21.77). Flavonoids content (mg/g) has shown following pattern in plants: *Alternanthera pungens* (79.26 ± 2.21) > *Trichodesma indicum* (78.6 ± 0.2) > *Adiantum incisum* (55.66 ± 8.02). Saponins (mg/g) were present in low quantities and presented the following order *Adiantum incisum* (16.13 ± 2.20) > *Trichodesma indicum* (5.2 ± 0.4) > *Alternanthera pungens* (4.4 ± 3.01). Total phenolics and tannins were determined by using gallic acid as standard. Straight line equations were obtained which were used to determine the total phenolics and tannins as gallic acid equivalents (GAE). Total phenolic and tannins content were determined by putting the value of absorbance in standard curve equation, where y is the absorbance of sample at 760nm and 725nm respectively and x is the total phenolics/ tannins content. Increase in total phenolics content ($\mu\text{g}/\text{mg}$ of GAE) in three plants were found to be in the following order: *Adiantum incisum* (133.96 ± 5.49) > *Trichodesma indicum* (76.53 ± 20.10) > *Alternanthera pungens* (43.45 ± 14.22) while total tannins content ($\mu\text{g}/\text{mg}$ of GAE) was found to be in this order: *Alternanthera pungens* (77.49 ± 34.10) > *Adiantum incisum* (60.82 ± 7.18) > *Trichodesma indicum* (46.05 ± 13.46).

Antitumor assay

Results (fig. 1 and table 2) showed that all extracts exhibited a pattern of high anti-tumor activity at high concentration which gradually decreases with decrease in concentration. Hexane extract of *Trichodesma indicum* was found to be the most effective extract showing significant antitumor activity at all concentrations while aqueous extract of *Adiantum incisum* was found to be the least effective extract showing relatively low antitumor activity at all the concentrations tested as compared to other extracts. Comparing the three extracts of three selected plants, following order of antitumor potential was revealed *Trichodesma indicum* > *Alternanthera pungens* > *Adiantum incisum*.

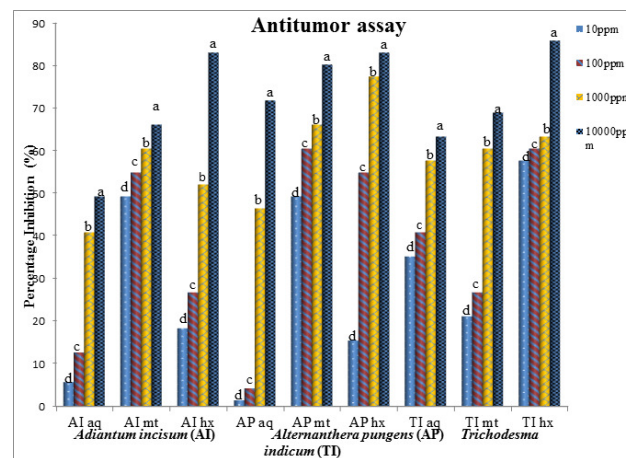
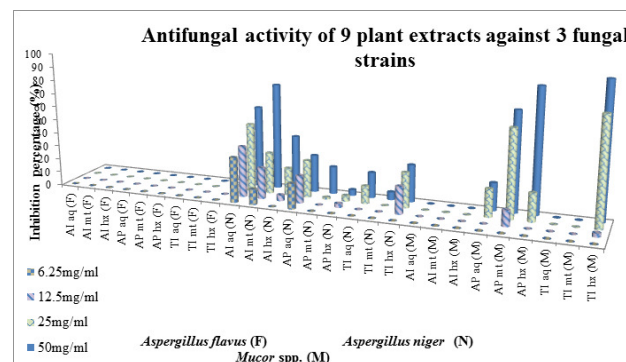


Fig. 1: Percentage inhibition of all plant extracts at different concentrations for antitumor assay. Different letters (a-d) show significant difference ($p < 0.05$) among the samples using ANOVA test. 100% tumor inhibition achieved with Vincristine.



100 [linear growth in samples (cm) / linear growth in control (cm)] $\times$ 100

Fig. 2: Percentage inhibition of plant extracts against three fungal strains. Inhibition% was calculated by using formula

Antifungal assay

All of the plant extracts showed no anti-fungal activity against *Aspergillus flavus*. Methanol extract of *Adiantum incisum* has shown significant anti-fungal activity (78.3%

inhibition) against *Aspergillus niger* while aqueous extract of *Adiantum incisum* has shown good activity (60.23% inhibition) against the aforementioned specie. Hexane extract of *Trichodesma indicum* has shown highest activity (98.9%) against *Mucor* spp. Results are depicted in fig. 2.

Antioxidant assays

Methanol extracts of all the three plant species have shown higher antioxidant potential (DPPH free radical scavenging activity) as compared to their respective aqueous and hexane extracts. Highest antioxidant activity was examined for *Adiantum incisum* methanol extract ($96.72\% \pm 1.48$) which were found to be very close to the activity of standard ($99.06\% \pm 0.06$) i.e. ascorbic acid, while lowest antioxidant activity ($24.59\% \pm 1.59$) observed in aqueous extract of *Alternanthera pungens*. All extracts of *Trichodesma indicum* depicted greater antioxidant potential as compared to other two plant extracts (fig. 3a).

Increase in absorbance is an indication of increase in reducing power of plant extracts. Highest values of absorbance were examined in aqueous extracts of *Adiantum incisum* (1.087 ± 3.2 - 0.309 ± 3.2) showing highest reductive potential among all plant extracts. Lowest reduction potential is attributed to aqueous extract of *Alternanthera pungens* because of the lowest absorbance values (0.168 ± 0.12 - 0.091 ± 0.132) among all plant extracts (fig. 3, b).

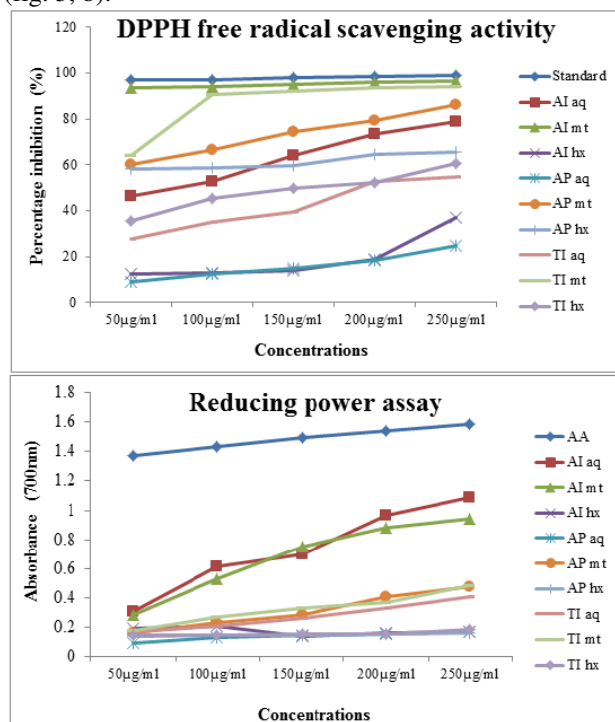


Fig. 3: DPPH free radical scavenging activity (a) and Reducing ability (b) of selected plant extracts at various concentrations in comparison with standard Ascorbic acid (AA).

DISCUSSION

Adiantum incisum, *Alternanthera pungens* and *Trichodesma indicum* were collected from Kund village and their aqueous, methanolic and n-hexane extracts were subjected to different bioassays which have not been carried out previously for these plants.

No previous study is available where *Adiantum incisum* was subjected to qualitative phytochemical analysis. Results of this research work are in agreement with Gracelin *et al.* (2013), who performed phytochemical analysis on five different species from the family Pteridaceae which includes *Pteris argyreae* T. Moore, *Pteris confusa* T.G. Walker, *Pteris vittata* L., *Pteris bialurita* L., and *Pteris multiaurita* Ag. and reported the presence of these phytochemicals in species of this family. Extracts of *Alternanthera pungens* also indicated various phytochemicals including alkaloids, flavonoids, saponins, tannins and terpenoids in this present study. Zongo *et al.* (2011) has reported the presence of saponins and steroids in aqueous extract of *Alternanthera pungens* which are also indicated in our study while the presence of alkaloids in present study and absence in previously reported study of Zongo *et al.* (2011) might be due to the difference in location of plant collection. Zongo collected the sample from Burkina Faso while our samples were collected from Kund village, Pakistan. Bama *et al.* (2013) qualitatively screened phytochemical components of 70% aqueous methanol extract of *Trichodesma indicum*. Research group reported the absence of alkaloids and presence of flavonoids and tannins in *T. indicum*. Our findings also confirm the said report. Most of the results of our present study for methanol extract of *Trichodesma indicum* are also in accordance with the results of Chidambaram and Aruna (2013) who also indicated the presence of saponins, tannins and flavonoids in methanolic extract of the said specie. Results of biochemical characterization of these plants also follows the same trend we previously reported using FTIR (Kazmi *et al.*, 2017) No reports in literature are available where quantitative estimation of these phytochemicals in any of the selected plant species is carried out. Gracelin *et al.* (2013) quantified these phytochemicals in five different species of the family Pteridaceae (family of *Adiantum incisum*). Alkaloids and flavonoids contents were higher in this family while saponins, phenolics and tannins content were lower in these five species. Present study also follows the same trend.

This is the first report where these plant extracts were employed for determination of antitumor potential. *Trichodesma indicum* is reported to have anticancer potential (Saboo *et al.*, 2013). It has been reported in many studies that flavonoids, phenols and tannins possess anticancer activity while flavonoids and its analogues have highest anticancer potential (Batra & Sharma, 2013).

Table 1: Qualitative (a) and quantitative (b) estimation of different phytoconstituents in selected plant species

Phytochemical Tests	<i>Adiantum incisum</i>			<i>Alternanthera pungens</i>			<i>Trichodesma indicum</i>		
	aq	mt	hx	aq	mt	hx	aq	mt	hx
Alkaloids	+	++	-	+++	-	-	+	-	-
Anthraquinones	-	-	-	-	-	-	-	-	-
Cardiac glycosides	+++	-	-	+	-	-	++	+	-
Coumarins	-	-	++	-	-	++	-	-	+++
Flavonoids	-	-	+	-	+++	+	-	+++	++
Glycosides	+++	+++	-	++	-	-	+++	++	-
Phenols	-	+++	+	-	+	++	-	+	++
Phlobatannins	-	++	-	+	-	-	-	-	-
Quinones	-	-	-	+++	-	-	++	+++	-
Saponins	-	+++	+	+++	-	+	+	++	-
Steroids	+	+++	-	+++	-	-	++	++	+
Tannins	+++	+++	++	-	+++	++	+++	-	+
Terpenoids	+++	+++	-	++	-	-	++	++	-

aq, mt and hx stands for aqueous, methanol and n-hexane extracts respectively

Strongly present +++, moderately present ++, weakly present +, Absent –

Plant name	Alkaloids (mg/g)	Flavonoids (mg/g)	Saponins (mg/g)	Total Phenolics (µg/mg)	Total Tannins (µg/mg)
<i>Adiantum incisum</i>	230.83 ^a ± 30.20	55.66 ^b ± 8.02	16.13 ^a ± 2.20	133.96 ^a ± 5.49	60.82 ^b ± 7.18
<i>Alternanthera pungens</i>	219.66 ^b ± 16.11	79.26 ^a ± 2.21	4.4 ^b ± 3.01	43.45 ^c ± 14.22	77.49 ^a ± 34.10
<i>Trichodesma indicum</i>	208.13 ^c ± 21.77	78.6 ^a ± 0.2	5.2 ^b ± 0.4	76.53 ^b ± 20.10	46.05 ^c ± 13.46

Different superscripts (a-c) show significant differences (P<0.05) among the samples using ANOVA test

Highest and lowest antitumor activity given by *Trichodesma indicum* and *Adiantum incisum* is in agreement with the flavonoid's contents determined in these plants. Extracts of *Trichodesma indicum* have shown strong presence of flavonoids, while extracts of *Alternanthera pungens* were intermediate and extracts of *Adiantum incisum* showed weak presence of flavonoids qualitatively. Quantitative estimation also indicated the lowest quantity of flavonoids in *Adiantum incisum*. Bibi *et al.* (2011) reported very significant antitumor potential by n-hexane fraction of *Aster thomsonii* using potato disc assay which is in agreement with present study as most significant results were obtained with n-hexane extracts of *Trichodesma indicum*. In another report maximum tumor inhibition was achieved by hexane extract as compared to methanol and aqueous extracts of *Euphorbia royleana* (Euphorbiaceae) (Ashraf *et al.*, 2015). Similarly hexane extract of *Artabotrys crassifolius* (Annonaceae) revealed potent anticancer potential using MTT assay (Tan *et al.*, 2014). These reports along with our findings suggest that non-polar compounds in plant extracts can be effective antitumor and anticancer agents.

All of the plant extracts showed no anti-fungal activity against *Aspergillus flavus*. Methanol extract of *Adiantum incisum* has shown significant anti-fungal activity (78.3% inhibition) against *Aspergillus niger* while aqueous extract of *Adiantum incisum* has shown good activity

(60.23% inhibition) against the aforementioned specie. Hexane extract of *Trichodesma indicum* has shown highest activity (98.9%) against *Mucor* spp. Results are depicted in fig. 2. Zongo *et al.* (2011) also reported no antifungal activity when using water, water acetone and water ethanol extract of *Alternanthera pungens* against fungal specie *Candida albicans*. Methanol extract of *Adiantum incisum* has shown significant activity against *Aspergillus niger* while aqueous extract of same plant has shown moderate activity. Terpenoids are reported to possess antifungal activity (Kashani *et al.*, 2012). Antifungal activity by *Adiantum incisum* extracts can be attributed to the strong presence of terpenoids. *Alternanthera pungens* and *Trichodesma indicum* extracts have very low or no activity against this fungal strain. Low activity of these plants corresponds to the moderate to weak level of terpenoids content in the respective plant species. Saboo *et al.* (2013) has reported moderate to significant antifungal activity by water and methanol extracts of *Trichodesma indicum* against *Aspergillus niger*. This difference might be due to the different part of the plant used in two studies. As Saboo *et al.* (2013) have used only aerial parts of *Trichodesma indicum* while in our present study; we have prepared whole plant extracts including roots. Roots of *Trichodesma indicum* present in our sample might play antagonistic effect for the said fungal specie. Another reason could be the different geographical locations of sample collection which are

responsible for different phytochemicals and thus different response.

Table 2: IC₅₀ of percentage inhibition by plant extracts for antitumor assay

Plant samples Extracts	IC ₅₀ values (ppm)		
	aqueous	methanol	hexane
<i>Adiantum incisum</i>	>10,000	12	540
<i>Alternanthera pungens</i>	1800	11	90
<i>Trichodesma indicum</i>	350	650	<10

Methanol extracts of all the three plant species have shown higher antioxidant potential (DPPH free radical scavenging activity) as compared to their respective aqueous and hexane extracts. Highest antioxidant activity was examined for *Adiantum incisum* methanol extract (96.72%±1.48) which were found to be very close to the activity of standard (99.06%±0.06) i.e. ascorbic acid, while lowest antioxidant activity (24.59%±1.59) observed in aqueous extract of *Alternanthera pungens*. All extracts of *Trichodesma indicum* depicted greater antioxidant potential as compared to other two plant extracts (fig. 3a). Good antioxidant ability was reported in hydro-alcohol extract of *Trichodesma indicum* (Dachani et al., 2012). Results of present study are in accordance with this report.

Increase in absorbance is an indication of increase in reducing power of plant extracts. Highest values of absorbance were examined in aqueous extracts of *Adiantum incisum* (1.087±3.2-0.309±3.2) showing highest reductive potential among all plant extracts. Lowest reduction potential is attributed to aqueous extract of *Alternanthera pungens* because of the lowest absorbance values (0.168±0.12-0.091±0.132) among all plant extracts (fig. 3, b). Higher value of absorbance is indication of higher reduction potential while higher reduction potential signifies higher antioxidant potential (Jayanthi & Lalitha, 2011). Dachani et al. (2012) found that reducing power ability of hydro-alcohol extract increases with increase in concentration of *Trichodesma indicum* extract. Present study also showed that reducing power ability of aqueous, methanol and *n*-hexane extracts of *Trichodesma indicum* also increases with the increase in concentration. Significant antioxidant potential by plant extracts is in accordance with the phytochemical analysis. Phenols and tannins have antioxidant abilities while the extracts of these plants were found rich in phenols and tannins. Highest antioxidant potential of methanol extract of *Adiantum incisum* is also in agreement with the fact that this plant species, *Adiantum incisum*, was richest in tannins. Tannins act as primary as well as secondary antioxidants. Various studies are reported which indicated significant antioxidant activity by tannins rich extracts (Amarowicz, 2007).

CONCLUSIONS

All three plants were found to be rich in important secondary metabolites which were responsible for significant antitumor and antioxidant potential as revealed by current research. Some extracts of these plants also possess good antifungal activity. This study elucidates further research for determining the antimicrobial, antioxidant and anti-cancer potential of these plants *in-vivo* and also promotes isolation and characterization of important secondary metabolites in these plants for production of pure therapeutic compounds.

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