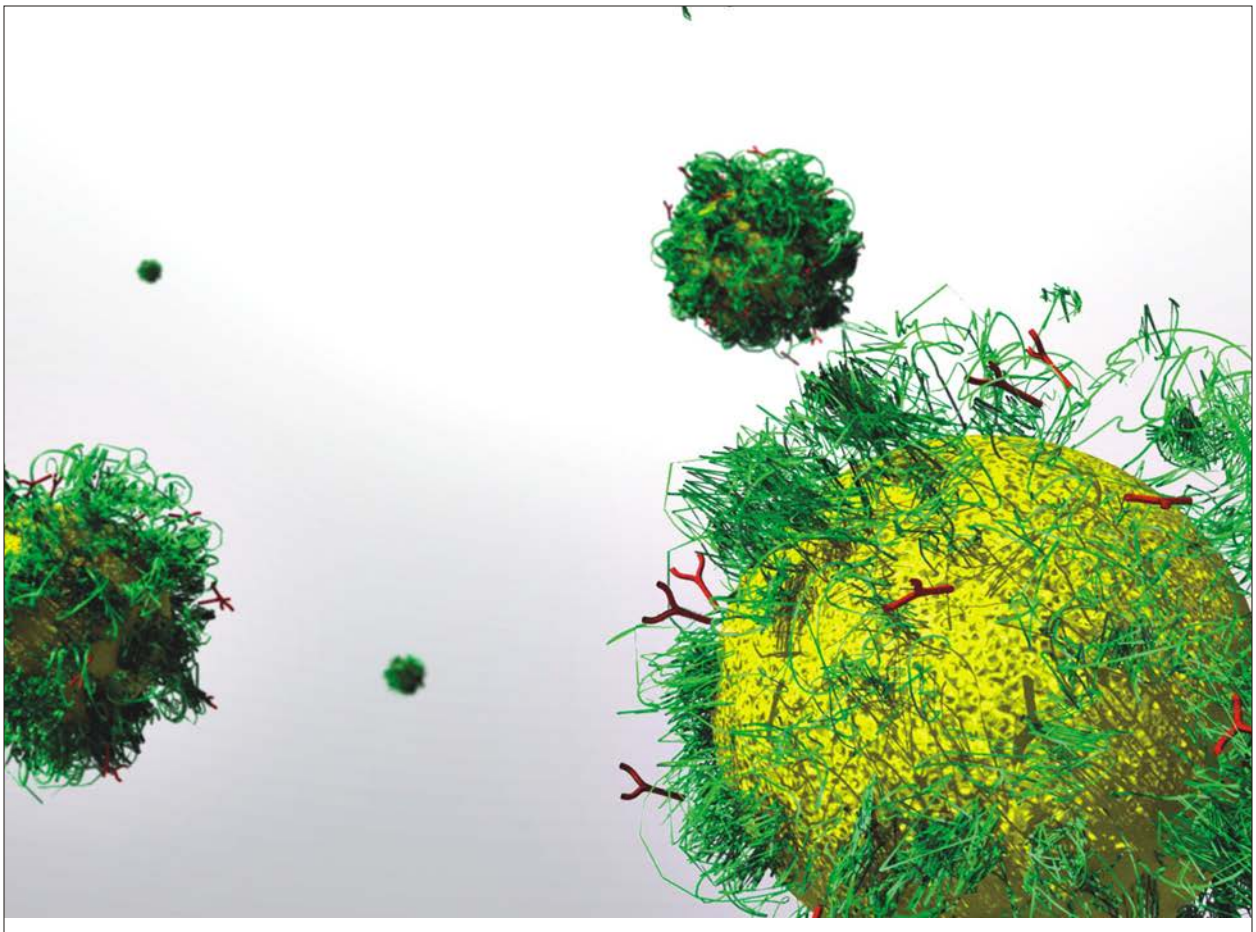

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Gynandropsis pentaphylla DC Extracts on the Production of Microbial Proteins

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ABSTRACT

The present study deals with the effect of *Gynandropsis pentaphylla* (leaves and seeds) extracts (acetone, benzene, methanol, ether and water) on the production of extra and intracellular protein in 6 bacteria and 4 fungi. Effects of different type of extracts were done through the estimation of intra and extra cellular proteins content. Acetone extract of seeds inhibited the extra cellular protein synthesis at maximum level (0.072±0.02 mg L^{-1}) in *Bacillus subtilis* NCIM 2010, followed by benzene extract of seeds which inhibited the synthesis of extra cellular protein (0.083±0.03 mg mL^{-1}) in *Escherichia coli* NCIM 2064. Water extract of seeds suppressed the extra cellular protein synthesis at maximum level (0.296±0.08 mg mL^{-1}) in *Aspergillus niger* NCIM 501. Intracellular protein synthesis was highly (0.285±0.12 mg mL^{-1}) affected after the addition of methanol extracts of leaves in *Staphylococcus aureus* NCIM 2120. Intracellular protein synthesis was highly (0.169±0.09 mg mL^{-1}) affected after the addition of benzene extracts of seed in *Penicillium notatum* NCIM 747. Sucrose, D-mannose and L-rhamnose were observed in the water extracts of leaves and seeds, when screened using TLC. Water extracts of leaves showed arginine, proline and methionine, while seeds showed proline and tyrosine.

Key words: Proteins synthesis inhibition, bacteria, *Aspergillus, Gynandropsis pentaphylla*, amino acids

INTRODUCTION

The leaves and seeds of cat's whiskers (*Gynandropsis pentaphylla* DC. (Capparidaceae)) are used in indigenous medicine in many countries (Chweya and Mnzava, 1997; Ajaiyeoba *et al*., 1998; Borgio *et al*., 2008a; Mule *et al*., 2008; Bala *et al*., 2010). In India, its common names include hurhur and karaila (Chweya and Mnzava, 1997). It is a herb indigenous to the tropical and sub tropical regions. The herb is edible (Borgio *et al*., 2008a; Mule *et al*., 2008) and has been used for several years traditionally in India for medical practices. *G. pentaphylla* leaves with a high percentage of vitamin C is taken as a pot herb in soups, fresh or dried. The leaves are used as disinfectants. Inhalation of the leaves also relieves headaches; leaf juice and oil, for earache and eye wash. Seeds have been reputed to have antihelmentic properties and oil is used as fish poison. Recently, the researcher has reported the antimicrobial properties of indigenous *G. pentaphylla* against 6 different bacteria and 4 fungi (Borgio *et al*., 2008a). *G. pentaphylla* plant has been

traditionally used as anthelmentic, rubefacient and is employed internally for expulsion of round worms and externally as counter-irritant and also used in cough and antiscorbutic, diaphoretic, emollient and applied to wounds, cobra bites. Leaves are applied externally to boils to prevent the formation of pus. Other ethnobotanical uses include carminative, malaria, piles and rheumatism and stomach tumor (abdomen). A decoction of the root is used to treat fevers (Mule *et al*., 2008; Bala *et al*., 2010). Also this plant has analgesic and anti-inflammatory activities (Mule *et al*., 2008).

Many pathogenic bacteria and fungi produce certain extracellular proteins (exotoxins) sufficiently potent that destroy certain cellular structures of the host or serious effects such as death often result in the host. No reports were available on the activity of *G. pentaphylla* against the extra and intra cellular protein synthesis in microorganisms. With these views in mind, the present study was designed to determine the capability of the leaves and seeds of *G. pentaphylla* to inhibit the protein synthesis in six bacterial and four fungal species such as *Acetobacter chrococcum, Bacillus subtilis, Lactobacillus acidophilus, Agrobacterium tumefaciens, Escherichia coli, Staphylococcus aureus, Aspergillus niger, Aspergillus flavus, Metarhizium anisopliae* and *Penicillium notatum*.

MATERIALS AND METHODS

Microorganisms: Pure cultures of *Bacillus subtilis* NCIM 2010, *Lactobacillus acidophilus* NCIM 2660, *Agrobacterium tumefaciens* NCIM 2145, *Escherichia coli* NCIM 2064, *Staphylococcus aureus* NCIM 2120, *Aspergillus niger* NCIM 501, *Aspergillus flavus* NCIM 650, *Metarhizium anisopliae* NCIM 1311 and *Penicillium notatum* NCIM 747 were obtained from the National Collection of Industrial Microorganism (NCIM), National Chemical Laboratory, Pune, India.

Acetobacter chrococcum GCITY 01 was purified from the mother culture in our laboratory.

Preparation of plant extract and reference compounds: *Gynandropsis pentaphylla* was collected from Rajgurunagar area of Pune, Maharashtra, India in March 2008. Extracts of leaves and seeds were prepared according to Borgio *et al.* (2008b) using water, acetone, benzene, methanol and ether. All the extracts were stored at 4°C for further analysis. Cloroamphenicol (10 mg mL^{-1}) and fluconozole (10 m mL^{-1}) (HiMedia, Mumbai, India) were used as references for bacteria and funi, respectively.

Estimation of extracellular proteins: Ten micro litter of 1% leaf extract was added to 10 mL of minimal broth. Sterile minimal broth was maintained as control. Minimal broths with plant extract, without plant extract and with standard reference compounds were inoculated with loopful of *E. coli* and incubated at 37°C for 24 h. The same protocol was repeated for all the bacteria and fungi. Fungal cultures were incubated at 27°C for 48 h. One hundred micro litter of cell free broth was taken along with 900 µl of distilled water. Optical density at 260 and 280 nanometers were recorded using minimal media as a blank in UV-Visible spectrophotometer (SL164) (Elico Ltd., India).

Concentration of proteins produced was calculated using the following formulae:

$$\text{Concentration} = (1.55\times A_{280})-(0.76\times A_{260})\ (\text{mg mL}^{-1})$$

Where:
A_{260} = Optical density at 260
A_{280} = Optical density at 280

Estimation of intracellular proteins: One sterile eppendorf tube was weighed using electronic balance (Shimadzu Corporation, Kyoto, Japan). The 1.5 L of culture (prepared as mentioned in the above paragraph) was centrifuged at 8000 rpm for 5 min and the supernatant was discarded. The above step was repeated thrice to get more quantity of cell pellet. The excess moisture was removed using tissue paper. The weight of the eppendorf tube along with pellet was taken once again to find the weight of the cell pallet. Five hundred micro litter of distilled water and 500 µL of 4M NaOH were added to lyse the cells for the release of their intracellular proteins. The content was mixed thoroughly and kept at 100°C for 5 min in water bath. The temperature was reduced to room temperature. Then OD was taken at 260 and 280 nm in UV-Visible spectrophotometer. The 2 M NaOH was used as a blank.

Concentration of proteins produced was calculated using the formulae mentioned above. The proportion index was calculated as given as:

$$\text{Proprotion index}\,(\%)=\frac{\text{Conc. of protein}}{\text{Weight of pellets}}\times 100\%$$

Thin layer chromatography: TLC was used for the separation of amino acids and sugars in the water extracts of leaves and seeds (Sadasivam and Manickam, 2004). Plate was developed in mobile phase using n-butanol: acetic acid: water (2:1:1) and n-butanol: acetic acid: water (8:2:2) for sugars and amino acids. Ninhydrin (0.1%) and 10% H_2SO_4 in methanol were spread to visualize the presence of amino acids and sugar respectively. Rf (Rf value is the ratio of distance travelled by the solute to the distance travelled by the solvent front) values were calculated for each amino acid and sugar.

Statistical analysis: Proportion indices of intracellular protein production by bacteria and fungi were calculated using the formula adopted by Borgio *et al.* (2008a). A t-test was conducted to determine statistical differences between the protein production by the pathogens after treatment with plant extracts and the protein production after treatment with the reference compound as well as the control without extract using STATISTICA/w 5.0. software.

RESULTS AND DISCUSSION

The effects of *Gynandropsis pentaphylla* extracts on production of extra cellular proteins in the bacteria and fungi tested are presented in Table 1 and 2, respectively. Acetone extract of seeds inhibited the extra cellular protein synthesis at the most significant level (0.072±0.02 mg mL^{-1}) in *Bacillus subtilis* NCIM 2010 followed by benzene extract of seeds (0.083±0.03 mg mL^{-1}) in *Escherichia coli* NCIM 2064 (Table 1). Among the fungi tested, water extract of seeds suppressed the extra cellular protein synthesis at maximum level (0.296±0.08 mg mL^{-1}) in *Aspergillus niger* NCIM 501 (Table 2). No extra cellular protein synthesis inhibiting ability was observed by the acetone and methanol extracts of leaves and seeds against *A. chrococcum* (Table 1). *Aspergillus niger* showed complete resistance towards all extracts of *Psidium guajava* at all concentrations (Ahmed and Yagoub, 2007). Similarly extract of *Verbesina encelioides* and *Solanum pseudocapsicum* also Iranian plants showed only appreciable activity against *A. niger* (Aliero *et al.*, 2006; Jain *et al.*, 2008; Keymanesh *et al.*, 2009). Recently, the effect of Onion (*Allium cepa*), Ginger (*Zingiber officinale*) and Garlic (*Allium sativum*) against *Aspergillus niger* showed higher activity, which is similar to the maximum protein synthesis inhibition in the present study (Tagoe *et al.*, 2011).

Table 1: *G. pentaphylla* extracts on production of extra cellular protein by bacteria

	Concentration of protein (mg mL^{-1})					
Extracts	*E. coli*	*S. aureus*	*B. subtilis*	*A. tumefaciens*	*L. aceidophilus*	*A. chrococcum*
CWE	0.167±0.02	0.246±0.06	0.521±0.06	0.328±0.05	0.301±0.02	0.412±0.06
CWA	0.085±0.07	0.258±0.05	0.371±0.20	0.464±0.08	0.356±0.01	0.499±0.04
Acetone seed	0.164±0.03*	0.399±0.02^{ns}	0.072±0.02**	0.442±0.06*	0.168±0.00**	0.442±0.04^{ns}
Acetone leaves	0.115±0.06*	0.241±0.06^{ns}	0.119±0.05**	0.275±0.04**	0.344±0.09*	0.774±0.10^{ns}
Methanol seed	0.159±0.05*	0.266±0.04^{ns}	0.670±0.25^{ns}	0.527±0.02^{ns}	0.261±0.09*	0.504±0.12^{ns}
Methanol leaves	0.374±0.06^{ns}	0.144±0.05**	0.372±0.01*	0.758±0.05^{ns}	0.481±0.08^{ns}	0.462±0.12^{ns}
Ether seed	0.145±0.05*	0.312±0.05^{ns}	0.531±0.06^{ns}	0.392±0.12*	0.284±0.04*	0.551±0.01^{ns}
Ether leaves	0.187±0.01^{ns}	0.273±0.03^{ns}	1.148±0.04^{ns}	0.275±0.06**	0.441±0.07^{ns}	0.491±0.06^{ns}
Benzene seed	0.083±0.03**	0.153±0.08**	0.549±0.05^{ns}	1.009±0.03^{ns}	0.292±0.05*	0.411±0.01*
Benzene leaves	0.141±0.06*	0.255±0.05^{ns}	0.631±0.09^{ns}	0.522±0.10^{ns}	0.293±0.04*	0.667±0.04^{ns}
Water seed	0.254±0.13^{ns}	0.253±0.07^{ns}	0.423±0.09*	0.720±0.00^{ns}	0.356±0.06*	0.577±0.05^{ns}
Water leaves	0.299±0.08^{ns}	0.278±0.08^{ns}	1.983±0.25^{ns}	0.518±0.00^{ns}	0.271±0.05*	0.528±0.02^{ns}

CWE: Control without extract ; CWA: Control with antibiotic, ^{ns} : Not significant. *,** :Significant at $p<0.05$ and $p<0.01$ as compared to controls following the t-test

Table 2: Effect of *G. pentaphylla* extracts on production of extra cellular protein by fungi

	Concentration of protein (mg mL^{-1})			
Extracts	*A. niger*	*A. flavus*	*M. anisopliae*	*P. notatum*
CWE	0.626±0.01	0.705±0.01	2.682±0.01	2.974±0.00
CWA	0.511±0.00	0.515±0.02	2.082±0.05	1.272±0.01
Acetone seed	0.782±0.04^{ns}	0.627±0.02*	1.585±0.04**	1.415±0.10*
Acetone leaves	0.4±0.02**	0.769±0.02^{ns}	2.122±0.00*	1.713±0.02*
Methanol seed	0.810±0.01^{ns}	0.673±0.03*	2.37±0.23*	0.792±0.06**
Methanol leaves	0.731±0.03^{ns}	0.724±0.04^{ns}	2.329±0.10*	0.618±0.03**
Ether seed	0.784±0.05^{ns}	0.626±0.08*	2.69±0.12^{ns}	0.943±0.01**
Ether leaves	2.263±0.13^{ns}	2.000±0.06^{ns}	2.135±0.10*	1.581±0.10*
Benzene seed	0.719±0.00^{ns}	8.657±0.20^{ns}	2.37±0.00*	1.387±0.09*
Benzene leaves	1.187±0.02^{ns}	0.743±0.00^{ns}	1.912±0.10**	1.425±0.08*
Water seed	0.296±0.08**	0.977±0.00^{ns}	2.37±0.00*	1.033±0.07**
Water leaves	1.100±0.01^{ns}	1.051±0.06^{ns}	2.37±0.01*	0.962±0.02**

CWE: Control without extract ; CWA: Control with antibiotic, ^{ns} : Not significant. *,**: Significant at $p<0.05$ and $p<0.01$ as compared to controls following the t-test

The impact of *G. pentaphylla* extracts on production of intra cellular proteins in the bacteria and fungi tested are presented in Table 3 and 4 respectively. Intracellular protein synthesis was most significantly (0.285±0.12 mg mL^{-1}) affected after the addition of methanol extracts of leaves to *Staphylococcus aureus* NCIM 2120 among bacteria and the addition of benzene extracts of seeds (0.169±0.09 mg mL^{-1}) to *Penicillium notatum* NCIM 747 among fungi. The total protein content (%) in cell pellet was calculated and described as proportion index of intra cellular protein production by bacteria and fungi, and are represented in Fig. 1 and 2, respectively. Organism (e.g., *P. notatum*) which had produced less protein (%) was highly inhibited by the specific extract (e.g., seed extract using benzene).

In the present investigation, the reduction in the protein synthesis in the bacteria and fungi after treatment with *G. pentaphylla* indicates that the plant extracts has significant potential to

Table 3: *G. pentaphylla* extracts on production of intra cellular protein by bacteria

	Concentration of protein (mg mL^{-1})					
Extracts	*E. coli*	*S. aureus*	*B. subtilis*	*A. tumefaciens*	*L. aceidophilus*	*A. chrococcum*
CWE	0.667±0.01	0.610±0.09	0.546±0.03	1.248±0.00	1.010±0.02	2.165±0.00
CWA	0.360±0.03	0.536±0.04	0.445±0.10	1.021±0.09	0.646±0.09	1.164±0.00
Acetone seed	0.586±0.08*	0.488±0.05**	0.984±0.05^{ns}	0.594±0.10**	0.925±0.09*	2.37±0.21^{ns}
Acetone leaves	0.513±0.00*	0.534±0.06*	0.313±0.06**	0.591±0.02**	0.940±0.04*	0.745±0.12**
Methanol seed	0.701±0.04^{ns}	0.420±0.02**	0.496±0.02*	0.678±0.03**	1.003±0.07*	0.724±0.05**
Methanol leaves	0.792±0.00^{ns}	0.285±0.12**	1.094±0.01^{ns}	1.244±0.20*	1.190±0.02^{ns}	1.583±0.14*
Ether seed	0.469±0.10*	0.513±0.08**	0.546±0.01*	1.350±0.00^{ns}	1.966±0.01^{ns}	1.147±0.10**
Ether leaves	0.821±0.23^{ns}	0.875±0.06^{ns}	0.783±0.03^{ns}	1.270±0.02^{ns}	1.925±0.01^{ns}	1.550±0.25*
Benzene seed	0.588±0.14*	0.412±0.08**	0.727±0.02^{ns}	1.806±0.09^{ns}	1.020±0.13^{ns}	1.940±0.01*
Benzene leaves	0.640±0.00*	0.688±0.07^{ns}	0.710±0.03^{ns}	1.303±0.12^{ns}	1.219±0.14^{ns}	1.978±0.12*
Water seed	0.788±0.05^{ns}	0.880±0.04^{ns}	0.849±0.00^{ns}	0.894±0.07**	0.944±0.12*	2.396±0.00^{ns}
Water leaves	0.652±0.09*	0.453±0.01**	0.993±0.00^{ns}	1.139±0.20*	0.858±0.00*	1.280±0.04*

CWE: Control without extract ; CWA: Control with antibiotic, ^{ns} : Not significant. *,**: Significant at $p<0.05$ and $p<0.01$ as compared to controls following the t-test

Table 4: Effect of *G. pentaphylla* extracts on production of intra cellular protein by fungi

	Concentration of protein (mg mL^{-1})			
Extracts	*A. niger*	*A. flavus*	*M. anisopliae*	*P. notatum*
CWE	1.154±0.02	1.226±0.05	1.031±0.01	0.893±0.08
CWA	0.628±0.04	0.594±0.07	0.745±0.02	0.668±0.04
Acetone seed	0.650±0.05*	0.654±0.01*	0.899±0.09*	0.606±0.09**
Acetone leaves	0.576±0.12**	0.723±0.10*	1.199±0.04^{ns}	1.166±0.06^{ns}
Methanol seed	0.631±0.00*	0.696±0.10*	0.481±0.08**	0.755±0.01*
Methanol leaves	0.786±0.03*	0.851±0.12*	0.803±0.04*	0.888±0.01*
Ether seed	0.591±0.01**	0.591±0.00**	0.361±0.01**	0.434±0.00**
Ether leaves	0.748±0.04*	0.723±0.00*	1.469±0.07^{ns}	0.752±0.00*
Benzene seed	0.581±0.09**	0.566±0.01**	1.358±0.10^{ns}	0.169±0.09**
Benzene leaves	0.794±0.07*	0.801±0.15*	0.448±0.04**	0.931±0.09^{ns}
Water seed	0.960±0.08*	0.979±0.12*	0.917±0.01*	0.653±0.05*
Water leaves	0.524±0.07**	0.527±0.01**	0.928±0.04*	0.705±0.01*

CWE: Control without extract; CWA: Control with antibiotic, ^{ns} : Not significant. * ,**: Significant at $p<0.05$ and $p<0.01$ as compared to controls following the t-test

alter the production of extra cellular proteins in all the organisms tested. Suppression of extra cellular protein synthesis at maximum level in *Aspergillus niger* using the water extract of seeds is interesting because water is one of the medium through which traditional healers utilize medicinal plants to their clients. Sudharhsan *et al.* (2007) reported the total protein content in *Bacillus* sp. In the current study, the quantity of proteins produced was more in the control than it is reported. Reports were not available on the effects of plant extracts on the protein production in bacteria and fungi.

Water extracts of leaves and seeds were screened using TLC for the presence of amino acids and sugars. The R_f values from TLC analysis indicated the presence of sucrose (0.1), D-mannose (0.2) and L-rhamnose (0.4) and gave black, brown and ligtht brown respectively, standards matching with the sugars at given R_f of standards. Silica gel 'G' plates were developed in mobile phase

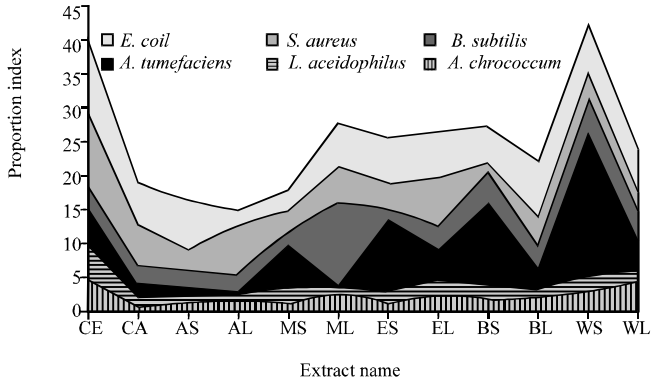

Fig. 1: Proprtion index of intra cellular protein production by bacteria. CE: Control without extract; CA: Control with antibiotic; AS: Acetone seed; Al: Acetone leaves; MS: Methanol seed; ML: Methanol leaves; ES: Ether seed; EL: Ether leaves; BS: Benzene seed; BL: Benzene leaves; WS: Water seed; WL: Water leaves

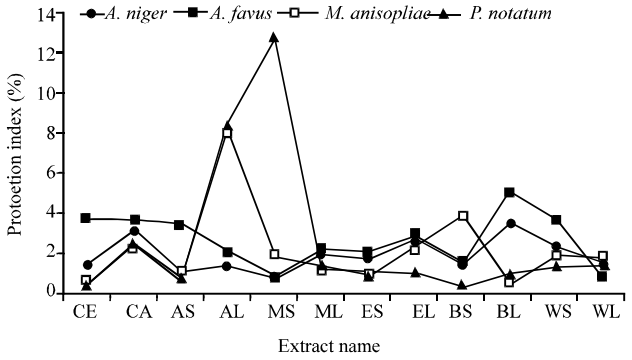

Fig. 2: Proprtion index of intra cellular protein production by fungi. CE: Control without extract; CA: Control with antibiotic; AS: Acetone seed; Al: Acetone leaves; MS: Methanol seed; ML: Methanol leaves; ES: Ether seed; EL: Ether leaves; BS: Benzene seed; BL: Benzene leaves; WS: Water seed; WL: Water leaves

n-butanol: acetic acid: water (2:1:1). The R_f values from TLC analysis of leaves showed the presence of amino acids such as arginine, proline and methionine, whereas the seeds contained proline and tyrosine (standard reference values).

In the current study, the R_f values from TLC analysis of leaves of *G. pentaphylla* showed the presence of amino acids such as arginine, proline and methionine, whereas the seeds contained proline and tyrosine. Borgio *et al.* (2008a) analysed the phytochemical constituents of Indian origin *G. pentaphylla,* they have reported the presence of secondary metabolites such as tannins, alkaloids, flavones, sugar, phenolic group, saponin, amino acid and essential oil in all the plant parts analysed. These secondary metabolites are known to exhibit medicinal activity as well as physiological activity (Ross, 2005). Borgio *et al.* (2008a,b) have also screened the effect benzene, methanol, acetone and ether extracts of various parts of *G. pentaphylla* against the growth of the microorganisms studied in the present research finding.

CONCLUSION

In the present study, all the organisms exhibited appreciable alteration in their protein production when treated with *G. pentaphylla*. Since microbial proteins play a vital role in pathogenesis, this study on the inhibition of protein production using *G. pentaphylla* would be of great value in drug designing against pathogens. Since this plant is easily available, their utilization makes possible the efficient exploitation of the local natural resource base. Further studies are needed to assess the activity of this plant against a broad spectrum of pathogens. Nevertheless, the analysis of chemical composition of the extracts with remarkable effect can direct to discovery of new antimicrobial substances which might be promising lead compounds for development of new synthetic molecules with antimicrobial activity.

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