Research Article

Antioxidant, Antibacterial and Anti-Proliferative Activity and Phytochemical Analysis of Selected Medicinal Plants from Dasapushpam of Kerala

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ABSTRACT

Kerala is endowed with rich biodiversity of medicinal plants which are widely used in various traditional medicinal systems like Ayurveda which are potential sources of bioactive constituents with effective and safer drugs. The present study is to evaluate the antibacterial, antioxidant and antiproliferative activity of traditional medicinal plants *Biophytum sensitivum* (L.) DC, *Curculiogo orchioides* Gaertn. and *Cynodon dactylon* (L.) Pers. The petroleum ether extract of *Curculiogo orchioides* showed significant antibacterial activity against pathogenic strains of bacteria. Among the other extracts methanolic extract of *Curculiogo orchioides* showed higher phenolic and flavonoid content and significant DPPH scavenging and reducing power activity. The antiproliferative activity was tested against Hep G2 (hepatocellular liver carcinoma) in which methanolic extract of *Biophytum sensitivum* and *Curculiogo orchioides* showed strong anticancer activity with an IC_{50} 108.72 and 127.12μg/ml respectively. Our study confirmed the ethanobotanical and traditional medicinal usage of the three medicinal plants *Biophytum sensitivum*, *Curculiogo orchioides* and *Cynodon dactylon*.

Keywords: Antibacterial, Antioxidant, Antiproliferative phytochemical analysis, Traditional medicinal plants.

INTRODUCTION

Traditional medicines have increasing demand in developed countries where western medicinal practices were long been standard. Traditional medicinal plants are the reservoirs of potential bioactive molecules. Recently developed 80% synthetic drugs have a natural compound prototype. The major advantages of Traditional medicine in comparison to allopathic medicine are the lesser side effect and cost effectiveness. Medicinal plants are excellent sources for the discovery of potential pharmacophores using structural and combinatorial chemistry. Ayurvedic medicinal system is a well established medicinal practice in India with a sound literature background originated approximately 5000 years ago.[1] Kerala is famous for its indigenous medicinal practice, Ayurveda and as the source of several potential medicinal plants. Dasapushpam constitute of ten medicinal plants which are culturally and therapeutically linked to folk of Kerala.[2] Science based approach is required to explore the biodynamic effect of these sacred plants.

Traditional medicinal plants are endless source for therapeutic drugs for various ailments like antimicrobial, anti-inflammatory, anticancer, antioxidants, antiulcer and so on. Despite tremendous progress in the field of antibiotics, Infectious diseases are still a major threat to the mankind. Development of antibiotic resistant strains adds the severity of the current scenario. Unexplored plant based antimicrobial agents as enormous scope for the development of safer and effective therapeutics.[3] Oxidative stress can be defined as the imbalance between the reactive oxygen species and the oxidative defensive mechanism of the body. Reactive free radicals are involved in the pathophysiology of various diseases like cancer, alzheimer's, cardiovascular diseases and aging etc.[4] Medicinal plants are potential sources for antioxidant compounds with different physical and chemical properties and mechanism of action.[5] Various plant derived antioxidants are effective free radical scavengers which are used combinatorial for the treatment of various diseases as nutritional supplements.[6] Malignancy is one of the most deadly diseases that affect human health in the modern world just below heart disease. About 60% of the chemotherapeutic drugs developed in recent decades are from plant origin. Medicinal plants are attractive sources for novel bioactive compounds having effective anticancer activity and lesser side effects.[7]

The present study is to evaluate antibacterial, antioxidant and antiproliferative activity of *Biophytum sensitivum* (L.) DC, *Curculiogo orchioides* Gaertn. and *Cynodon dactylon* (L.) Pers. which are considered to be three important medicinal plants of Dasapushpam.

MATERIAL AND METHODS

Plant collection and extraction

Biophytum sensitivum (BS) *and Cynodon dactylon* (CD) whole plant and *Curculiogo orchioides* (CO) rhizomes were collected from local medicinal market of Thrissur, Kerala. The specimens were authenticated by the plant biotechnology division of VIT University, Vellore. The samples were washed thoroughly, chopped into small pieces and shade dried. The samples are pulverized in an electric blender and the powdered samples are used for further extraction. Soxhlet extraction was done for each sample sequentially using the solvents petroleum ether

(PE) dichloromethane (DM), methanol (ME) and aqueous (AQ) in the increasing order of polarity. All the extracts were concentrated using rotary evaporator and the extracts were stored in air tight container until use.

Phytochemical screening

Preliminary phytochemical screening of the four extracts of *Biophytum sensitivum* (BS), *Cynodon dactylon* (CD) and *Curculiogo orchioides* (CO) were performed by Trease and Evans [8] and Harborne.[9]

Total phenolic content

The total phenolic content was determined by using Folin-Ciocalteu assay.[10] The extracts were oxidized with Folin-Ciocalteu reagent, and the reaction was neutralized with sodium carbonate. 50µl of sample or standards were mixed with 2.5ml of 1:10 diluted Folin-Ciocalteau's reagent and to which 2ml of Na_2CO_3 (7.5% w/v) were added. The Folin-Ciocalteu reagent is a yellow colored acid consisting of acid polyhetero rings containing phosphotungestic acid ($H_3PM_{12}O_{40}$) and phophomolybdic acid ($H_3PW_{12}O_{40}$) which would be oxidized by the phenolic compounds of the extract to form stable blue complex: molybdene(Mo_8O_{23})-tungstene (W_8O_{23}) which has maximum absorbance at 760 nm. The total phenolic content was expressed in terms of mg gallic acid equivalent per g of extract. Experiments were performed in triplicates.

Total flavanoid content

Total flavonoids content was determined spectroscopically by the aluminium trichloride method[11] with slight modification using quercitin as standard. 0.5ml of extracts or standards and 3ml of distilled water were added to the volumetric flask. 0.3ml of 5% $NaNO_2$ was added to the flask at zeroth minute. After 5min 0.3ml 10% $AlCl_3$ and incubated at RT. After 6 minute 2ml of 1M NaOH were added and the final volume made up to 10ml. The absorbance was measured at 510 nm and the results were expressed in mg quercitin equivalent per gram of extract. Experiments were performed in triplicates.

Antibacterial activity

Microorganism and culture conditions

The bacterial strains used for the study includes: *Streptococcus pyogenes, Staphylococcus aureus, Bacillus coagulans, Bacillus lichenformis, Bacillus cereus, Bacillus subtilis and Clostridium* for Gram positive, *Escherichia coli, Proteus mirabilis and Pseudomonas fluorescens* for Gram negative microorganisms. Strains used for the study were obtained from the National Collection of Industrial Microorganisms (NCIM), Pune, India, and Microbial Type Culture Collection (MTCC), Chandigarh, India. All the microbial strains were revived from glycerol stock at -80°C. After bringing it at room temperature nutrient broth was added into the cultural vials. The cultures were sub-cultured for activity assays in nutrient broth by incubating at 37°C for 24 hours and stock was prepared and stored at 4°C.

Antibacterial assay with well diffusion method

The microbial growth inhibitory potential of the extracts of *Biophytum sensitivum* (BS), *Cynodon dactylon* (CD) and *Curculiogo orchioides* (CO) were determined by using the agar well diffusion method.[12,13] The pre-inoculated cultures were made to the turbidity of 0.5 McFarland standard turbidity (106 CFU/ml) and these bacterial inoculums were uniformly spread on the media using a sterile cotton swab. Wells (9mm) were made into the media using a sterile cork borer. Extracts were dissolved in 10% DMSO to a final concentration of 1mg/ml. One hundred microliters of the extracts were transferred into the well. Kanamycin (10µg/ml) was used as positive drug control and 10% DMSO was used as solvent control. The systems were incubated at 37°C for 24 hours and zones of inhibition were measured in mm after the incubation.

MIC determination

The minimum inhibitory concentration (MIC) of the extracts was determined for the test organisms on which the plant extracts showed potent antibacterial activity.[14] The Minimum Inhibitory Concentration Assay is a technique used to determine the lowest concentration of a particular antibiotic needed to kill bacteria. To 2 mL of nutrient broth was added 1 mL of varying concentration of the extracts and serially diluted to obtain the following final concentrations of extracts: 500 mg/L, 250 mg/L, 125 mg/L, 62.5 mg/L, 31.25 mg/L, 15.62 mg/L, 7.81 mg/L, 3.90 mg/L, 1.95 mg/L and 0.097 mg/L. Afterwards, 1mL of the test organism was introduced to the tubes. A tube containing nutrient broth only was seeded with the test organisms as described above to serve as control and another one was containing only broth to serve as blank. Tubes containing bacterial cultures were then incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration (mg/L) of the extract in the tubes showing no visible bacterial growth.

Antioxidant activity

DPPH assay

The DPPH assay was performed to determine the free radical scavenging potential of the extracts[15]. 1ml of 0.1 mM DPPH in methanol was mixed with 3ml of different concentrations of extracts and standards. The solution is mixed vigorously and incubated in darkness for 30min. The free radical (1,1-diphenyl-2-picrylhydrazyl) which is absorbing UV-light at 517 nm will be reduced in the presence of antioxidant compounds contained in the extract. This reaction will form a yellow molecule which will not absorb at the working wavelength. The more potential the extract is, the higher free radical scavenging i.e., the lower absorbance at 517 nm is measured.

The percentage of scavenging was calculated as follow:

$$\% \text{ Scavenging} = (1 - A_{sample}/A_{control}) \times 100$$

Where A_{sample} is the absorbance measured in the presence of extract and $A_{control}$ is the one measured in absence of extract.

Nitric oxide radical scavenging

Nitric oxide scavenging activity of the extracts was determined using the method of Ebrahimzadeh[16] with slight modification.1ml of 10 mM sodium nitroprusside was mixed with 1ml of different concentration of extracts and standards and the mixture was incubated at 37°C for 150min. After incubation 1ml of the mixture was taken out to which 1ml of Griess' reagent (1% sulphanilamide and 0.1% naphthyl ethylene diamine dihydrochloride in 2% o-phosphoric acid) was added and the absorbance were measured at 546nm. The procedure is based on the principle that, sodium nitroprusside (SNP) in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions. These nitrite ions can react with Griess reagent and to form a chromophore absorbing at 546 nm. Scavengers of nitric oxide (present in the extract) compete with oxygen, leading to decrease in the production of nitrite ions. The absorbance of the chromophore which was formed will be measured at 546 nm and will decrease in presence of extract.

The percentage of scavenging was calculated as follow:

$$\% \text{ Scavenging} = (1 - A_{sample}/A_{control}) \times 100$$

Where A_{sample} is the absorbance measured in the presence of extract and $A_{control}$ is the one measured in absence of extract.

Total antioxidant activity

Total antioxidant activity of the extracts was determined according to the procedure of Sonia[17] with slight modification. 0.3ml of extracts was mixed with 3ml of the reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated at 95°C for 90min. After incubation tubes were cooled and the absorbance was measured at 695nm. The phosphomolybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by antioxidant compounds present in the sample and the subsequent formation of green phosphate/Mo (V) complex at acid pH, which absorbs at 695 nm. Total antioxidant activity is expressed as ascorbic acid equivalent/g of extract.

Reducing power assay

The reducing power assays for the extracts were performed using the method Kalaivani.[18] 1ml of different concentrations of extracts was mixed with phosphate buffer (2.5 ml, 2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%) and the mixture was incubated at 50°C for 20 min. 2.5 ml of trichloroacetic acid (TCA, 10%) was added to the mixture which was then centrifuged at 5000rpm for 15min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Substances, which have reducing potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which in turn react with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. Thus if the sample has antioxidant properties, the absorption at 700 nm will increase with the concentration of extract.

FRAP assay (Ferric Reducing Antioxidant Power)

The principle of the FRAP assay is that antioxidants containing in the extract reduce Fe^{3+}/tripyridyltriazine complex to the blue colored ferrous form, with an increase in absorbance at 593 nm.[19] The reaction mixture (3ml) was prepared by adding 2.85ml of FRAP reagent and 0.15ml of extracts and incubated for 30min at 37°C in the water bath. Absorbance was taken immediately after incubation at 593nm using acetate buffer as blank. Standard calibration curve of Fe (II) concentration from 100-1000 µmol/L $FeSO_4$ $7H_2O$ was prepared and the results were expressed as ferric-TPTZ reducing ability equivalent to that of µmol Fe (II)/ g extract.

Cell culture and treatment

Human cancer cell lines HepG2 (hepatocellular liver carcinoma) were obtained from National Centre for Cell Sciences (NCCS), Pune. Cells were maintained in DMEM media supplemented with 10% FBS 100 U/ml penicillin and 100 µg/ml streptomycin with 5% CO_2 at 37°C in CO_2 incubator. The cultured cells were harvested, counted and used for further assays.

Anti-proliferative activity

The cytotoxicity of the methanolic extracts of *Biophytum sensitivum* (BS), *Cynodon dactylon* (CD) and *Curculiogo orchioides* (CO) were measured against human cancer cell lines (breast cancer), HepG2 (hepatocellular liver carcinoma) with the MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium) assay, MTT assay is based on the cleavage of a tetrazolium salt by mitochondrial dehydrogenases in viable cells to form blue formazan product.[20] Hundred microliters of the cell suspension of 5×10^3 cells/well were seeded into a 96 well plate and 100µl of the extracts at various concentrations (50-300 µg/ml) and positive control (doxorubicin) were added to the wells and the plates were incubated for 48hr in a CO_2 incubator. After the incubation period 20 µl MTT (5 mg/ml) was added to each well and kept for 2 hr incubation. The insoluble formazan crystals formed were solubilised by the addition of 100 µl MTT lyses buffer followed by an incubation of 4 hr and the plates were read at 570 nm using microtitre plate spectrophotometer.[21,22] The inhibitory rate was calculated as follows:

Inhibitory rate (Ir) % = 100 – Proliferation rate (Pr)

Proliferation rate (Pr) % = [Abs sample / Abs control] × 100

RESULTS AND DISCUSSION

Phytochemical analysis

Preliminary qualitative analysis of the four extracts confirmed the presence of phyto constituents like tannin, phenolics, flavanoids, steroids, glycosides, terpenoids,

and reducing sugar in various extracts of *Biophytum sensitivum*. The methanolic extract of *Cynodon dactylon*, was rich with wide range of plant compounds like phenolic, tannin, saponin, flavonoids, terpenoids, glycosides and sugars. The presence of phenolics, tannin, steroids, flavonoids, terpenoids and glycosides were confirmed in the methanolic extract of *Curculiogo orchioides* (Table 1).

Table 1: Phytochemical analysis of *Biophytum sensitivum*, *Cynodon dactylon* and *Curculiogo orchioides*

	Cynodon dactylon				*Curculigo orchioides*				*Biophytum sensitivum*			
	PE	DM	ME	AQ	PE	DM	ME	AQ	PE	DM	ME	AQ
Tannin	-	-	+	-	-	-	+	-	-	-	+	-
Phenol	-	-	+	-	-	-	+	-	-	-	+	-
Saponnin	-	-	+	+	-	-	-	+	-	-	-	+
Flavonoids 1	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	-	-	-	-	-	+	-	-	-
3	+	+	+	-	-	-	-	-	+	-	-	-
Steroids	+	+	-	-	-	-	+	-	-	-	+	-
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-
Cardiac Glycosides 1	+	+	+	-	+	+	+	-	+	+	-	-
2	-	+	+	-	-	+	+	-	+	+	-	-
Terpenoids	-	-	-	-	-	-	+	-	-	+	-	-
Carbohydrates 1	-	-	+	-	-	-	+	+	-	-	+	-
2	-	-	-	-	-	-	-	+	-	-	-	-
3	+	-	-	-	-	-	-	-	+	+	-	-
Proteins	+	+	-	-	-	-	-	-	-	-	-	-
Reducing sugar	+	+	-	-	-	-	-	-	+	+	-	-

Table 2: Total phenolic and flavonoid content of methanolic extracts of *Biophytum sensitivum*, *Cynodon dactylon* and *Curculiogo orchioides*.

Methanolic extract	Total phenol content (in mg eq. of GA/ g of extract)	Total flavonoids content (in mg of quercitin eq. / g of extract)
Curculigo orchioides	537±2.6	400±5.5
Cynodon dactylon	453±2	186±7.0
Biophytum sensitivum	485±1.7	248±3.1

Table 3: Zone of inhibition of antibacterial activity of *Biophytum sensitivum*, *Cynodon dactylon* and *Curculiogo orchioides*.

	Standard	CD		CO		BS
	Kanamycin	PE	ME	PE	ME	ME
Streptococcus pyogenes	13	-	-	13	5	7
Staphylococcus aureus	15	-	-	10	12	11
Bacillus coagulans	19	6	6	8	6	7
Bacillus licheniformis	12	-	-	13	-	-
Bacillus cereus	18	-	4	11	-	5
Bacillus subtilis	17	-	-	7	-	-
Clostridium	14	3	-	-	-	-
Proteus mirabilis	23	6	-	10	-	-

Total phenolic and flavanoid content

The total phenolic content of the methanolic extracts were determined using the linear regression equation of the gallic acid calibration curve ($y=0.0507x-0.269$; $R^2=0.9812$) and then the total phenol content is expressed as mg equivalent of gallic acid per gram of extract. The total flavanoid content of the extracts were expressed as mg of quercitin equivalent per gram of extract (Table 2). The methanolic extract of *Curculiogo orchioides* showed highest flavonoid and phenolic content, 400±5.5mg quercitin equivalence/g of extract and 537±2.6mg gallic acid equivalence/g of extract.

Table 4: Minimum Inhibitory Concentration (MIC) of methanolic extract of *Biophytum sensitivum* and petroleum ether extract of *Curculiogo orchioides*.

Plant extract	*Curculigo orchioides, petroleum ether*	*Biophytum sensitivum, methanol*
Streptococcus pyogenes	1.95	15.62
Staphylococcus aureus	3.90	7.81
Bacillus coagulans	7.81	15.62
Bacillus cereus	1.95	31.25

Table 5: Total antioxidant and Ferric Reducing Antioxidant Power assay of methanolic extracts of *Biophytum sensitivum*, *Cynodon dactylon* and *Curculiogo orchioides*.

Methanol extract	mg equivalent ascorbic acid/ g of extract	FRAP (µmol Fe(II)/ g of extract
Biophytum sensitivum	22.47±0.5	119±1.1
Curculigo orchioides	38±0.64	45±2.3
Cynodon dactylum	25±0.50	147±3.7

Antibacterial activity

Agar well diffusion method is the widely accepted method for the evaluation of antibacterial activity of samples.[23] Preliminary screening for the antibacterial activity of the extracts was performed with various strains of gram +ve and –ve pathogenic bacterial strains. Among the four extracts methanolic extracts of three medicinal plants showed potent inhibition. Petroleum ether extracts of *Cynodon dactylon* (CD) and *Curculiogo orchioides* (CO) also showed significant inhibition. For the further studies only the samples which showed positive results were tested for the antibacterial activity. Among the five extracts, petroleum extract of *Curculigo orchioides* showed best potential activity against the bacterial strains used. The methanolic extracts of *Curculigo orchioides* and *Biophytum sensitivum* also showed important inhibitory activity against bacterial cultures. Most effective activity was exhibited by *Curculigo orchioides*, petroleum ether extract against *Streptococcus pyogenes* and *Bacillus licheniformis* with a zone of inhibition of 13mm, which was almost equivalent to the standard antibiotic (Table 3). The MIC values of the methanolic extract of *Biophytum sensitivum* ranged from 7.81 to 31.25 mg/L. The most significant inhibition of bacterial growth was shown by petroleum ether extract of *Curculiogo orchioides* with an MIC value of 1.95mg/L against *Staphylococcus aureus* (Table 4). The major phytoconstitiuents present in the petroleum ether extract of *Curculiogo orchioides* is fatty acids, hence the potential antibacterial activity against the pathogenic strains shown by the extract may be attributed to the fatty acids present in it.[24]

Antioxidant activity

Free radicals are molecules or atoms that have at least one unpaired electron which usually increases the chemical reactivity of the molecule. Free radicals can react with other molecules to cause cell damage or DNA mutation.[25] Molecules called antioxidants protect against free radical damage and their action permit to ensure a balance between production and destruction of free radicals.[26] Antioxidant capacity of the methanolic extracts of *Biophytum sensitivum* (BS), *Cynodon dactylon* (CD) and *Curculiogo orchioides* (CO) were evaluated by several antioxidant assays. DPPH radical scavenging assay shows that the methanolic extracts of the three plants have potential antioxidant activity which increases with the concentration of the extract. *Curculiogo orchioides* (CO) seems to be the most potential extract against DPPH radical with the lowest Effective Concentration which scavenges 50% radical (EC_{50}) of 23 µg/mL (Figure 1). Nitric oxide is a major free radical generated in the body during biological metabolism which is directly involved in the pathophysiology of various diseases like inflammation and cancer.[27] The methanolic extract of of *Biophytum sensitivum* (BS) showed significant nitric oxide scavenging activity in a dose dependent manner (Figure 2).

The total antioxidant capacity in extract was determined using the linear equation of the calibration curve ($y = 0.0099x + 0.0447$; $R^2 = 0.9842$) and was expressed as the number of equivalent of ascorbic acid/g of extract. The methanolic extract of *Curculiogo orchioides* (CO) showed highest total antioxidant capacity of 38 mg equivalent of ascorbic acid/g of extract (Table 5). The reducing ability of the extracts is an indicator of potential antioxidant activity which can terminate the free radical chain reaction by converting them to stable products.[28] The reducing capacities of the extracts were determined by reducing power assay and FRAP assay.[29] Among the three plants methanolic extract of *Biophytum sensitivum* (BS) showed significant reducing potential in a dose dependent manner (Figure 3). The increase in the absorbance indicates the higher reducing power. The methanolic extract *Curculiogo orchioides* (CO) showed highest FRAP antioxidant activity 45 µmol Fe(II)/ g of extract followed by *Biophytum sensitivum* (BS) and *Cynodon dactylon* (CD), 119 and 147 µmol Fe(II)/ g of extract respectively (Table 5). Looking back to the phytochemical results of our extracts, methanolic extract is the effective solvent for extraction of flavanoid and

phenolic compounds which is in agreement with results of Samia[30] The total phenolic and flavanoid content of the methanolic extract of *Curculiogo orchioides* found to be higher compared to *Cynodon dactylon* and *Biophytum sensitivum* which correlates to the potential antioxidant activity of the extract. The plants having higher amount of polyphenolic compounds like flavanoids and phenolics are potential free radical scavengers which are effective in prevention of various diseases.[31,32]

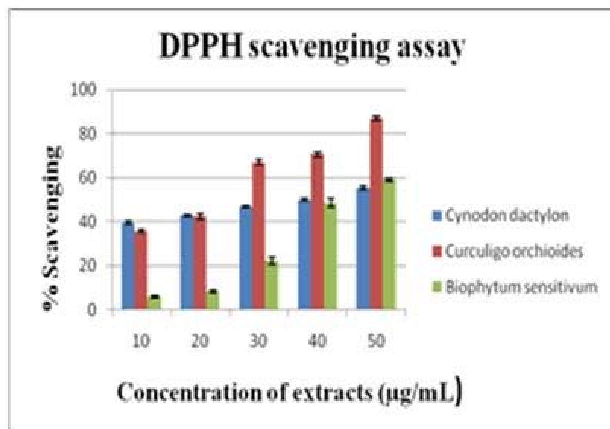

Figure 1: DPPH radical scavenging assay of methanolic extracts of *Biophytum sensitivum*, *Cynodon dactylon* and *Curculiogo orchioides*.

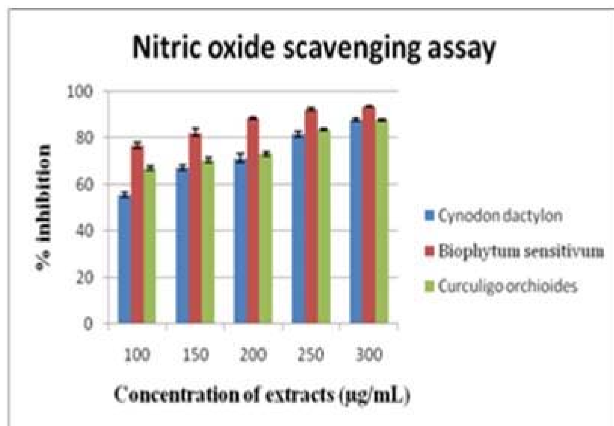

Figure 2: Nitric oxide radical scavenging assay of methanolic extracts of *Biophytum sensitivum*, *Cynodon dactylon* and *Curculiogo orchioides*.

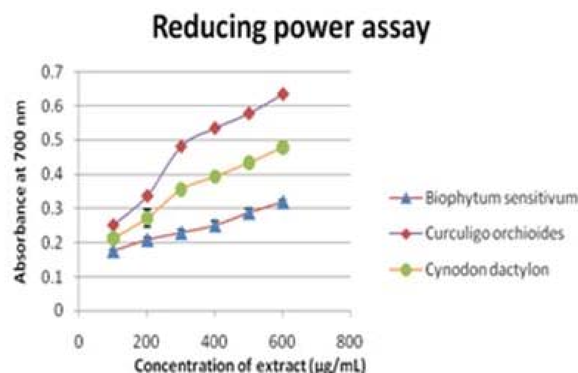

Figure 3: Reducing power assay of methanolic extracts of *Biophytum sensitivum*, *Cynodon dactylon* and *Curculiogo orchioides*.

Anti-proliferative activity

Even though there is remarkable development in the field of molecular mechanism of cancer, the development of chemotherapeutic agents still remains ineffective and costly.[33] Medicinal plants showing potential activity are important sources of bioactive molecules which can be developed as potent chemotherapeutic agents.[34] The cytotoxicity of *Biophytum sensitivum* (BS), *Cynodon dactylon* (CD) and *Curculiogo orchioides* (CO) was measured against human cancer cell lines HepG2 cells using the MTT (3-[4, 5- dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium) assay. Different concentrations (50-250µg/ml) were used for the assay. 10% DMSO was used as negative control and Doxorubicin as positive control. Compared with the *Cynodon dactylon* (CD), methanolic extract of *Biophytum sensitivum* and *Curculiogo orchioides* showed potent activity in a dose dependent manner (Figure 4). Methanolic extract showed significant cytotoxicity activity with an IC_{50} 108.72 and 127.12µg/ml for *Biophytum sensitivum* and *Curculigo orchioides* respectively at 48hr. Significant amount of phenolic content were found to be present in the methanolic extract of *Biophytum sensitivum* and *Curculigo orchioides* which may be involved the anticancer activity of the extracts. Further studies are required to identify the potential compounds and their mechanism of action.

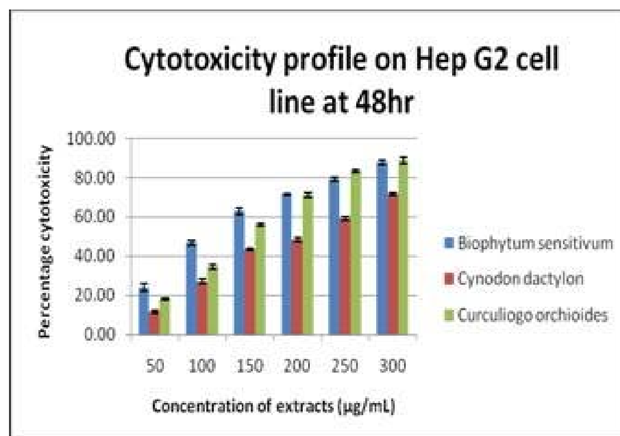

Figure 4: Cytotoxicity profile of methanolic extracts of *Biophytum sensitivum*, *Cynodon dactylon* and *Curculiogo orchioides* on HepG2 cell line at 48hr.

CONCLUSION

In conclusion, methanolic extracts of *Biophytum sensitivum* (BS), *Cynodon dactylon* (CD) and *Curculiogo orchioides* (CO) found to be more potential compared to the other extracts which may be attributed to the high phenolic and flavanoid content of the extract. *Curculiogo orchioides* and *Biophytum sensitivum* showed significant antibacterial, antioxidant and anticancer compared to *Cynodon dactylon*. Further studies are required to isolate and characterize the bioactive compounds and their mechanism of action which may lead to the development of novel compounds. So, it is anticipated that plants *Biophytum sensitivum* Linn and *Curculigo orchioides*

Gaertn can provide potential bioactive compounds for the development of new 'leads' to combat cancer diseases.

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