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Phytochemical analysis and *in vitro* antioxidant capacity of different solvent extracts of *Saussurea lappa* L. roots

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Abstract
In the studies phytochemical analysis, estimation of total phenolic and flavonoid content and antioxidant potential of costus roots was evaluated. Different solvent extracts were prepared using soxhlet extractor and roots were extracted by different solvents separately. Results revealed that methanolic extract of the roots was most active among all the tested root extracts. It contains highest phenolics and flavonoid content and maximum number of phytochemicals were detected in the methanolic extract. The amount of phenols and flavonoids varied from 12.34 to 75.02 mg GAE/g and 16.2 to 67.60 mg QE/g respectively. High antioxidant activity of methanolic extract can be related to the high amount of phenols and flavonoids. Although they were less effective than ascorbic acid standard but may provide a good source for new natural antioxidants.

Keywords: Antioxidant capacity, costus roots, phytochemical, *Saussurea lappa* L. solvent extracts

Introduction
Plant-derived drugs remain an important resource, especially in developing countries, to combat serious diseases. Approximately 60-80% of the world's population still relying on traditional medicines for the treatment of common illnesses [1, 3]. There are about 60-90% of patients with arthritis who have used complementary and alternative medicine but most used traditional Chinese medicine. The use of medicinal plants as a source for relief from illness can be traced back over five millennia to the written documents of the early civilization in China, India and the near East, but it is doubtless an art as old as mankind [4]. Although, the potential of higher plants as source for new drugs is still largely unexplored. Over the past decades, there has been increasing interest in the investigation of the natural products from different sources particularly from higher plants for the discovery of new antimicrobial and antioxidant agents, such as tannins, terpenoids, alkaloids, and flavonoids, which have been demonstrated to have *in vitro* antimicrobial properties [5, 9]. As this may give a new source of antimicrobial agents, many research groups that are now engaged in medicinal plants research have given much attention to these natural resources.

Saussurea lappa belongs to the family Asteraceae (Compositae) is commonly known as Costus. It is the largest family of dicotyledons, comprising 950 genera and 20,000 species, out of which 697 species occur in India. They are worldwide in distribution and abundance in the tropics, cold arctic or alpine regions [10]. It is an erect robust perennial herb 1- 2 cm. tall, apparently, at altitude of 25,500-3,000 meter, and also cultivated in Kashmir and neighbouring Himalayan regions for its roots used in medicine. Root stout, often up to 60 cm long, possessing a characteristic penetrating odour; stem stout, fibrous; radicals leaves with long lobately winged stalk, up to 1m long; flower heads stalk less, very hard, rounded, 3-5 cm. in diameter, flowers dark blue-purple or almost black, in axillary and terminal clusters; achene 3 mm. long, curved, compressed [11]. Kuth is also commonly known as Costus in trade has however no connection with the botanical genus Costus. It is found growing wild only in Jammu and Kashmir in the Kishenganga valley and the higher elevations of the Chenab valley, although it may occur sporadically in non-commercial quantities in adjoining tracts in Kashmir and elsewhere. The plant has become almost extinct in many places by uncontrolled exploitation. Since the supply of plants growing wild was not sufficient to meet the market requirement, commercial cultivation of Kuth was taken up during the second and third decades of this century in its natural growing areas in Kashmir, Lahul in Himachal Pradesh and Garhwal in Uttar Pradesh. It has been successfully cultivated in semi-natural conditions in the forest areas in Kashmir and Garhwal [12, 13]. The roots contain odorous principles composed of two liquid resins, an alkaloid, a solid resin, salt of valeric acid an astringet principal and ash

containing manganese. The costus root oil has the following composition:- Camphene (0.04%), phellandrene (0.4%), terpene alcohol (0.2%), □-costene (6.0%), aplotaxene (20.0%), costol (7.0%), di-hydrocostus lactone (15.0%), costus lactone (10.0%) and costic acid (14.0%). Active principal of the root are: Essential oil of a strong aromatic penetrating and fragrance odour (1.5%); aglucoside and an alkaloid Saussurine (0.05%). Kuth roots contain resinoids (6%), and essential oil (1.5%), alkaloid (0.05%), inulin (18%), saussurea lactone (20-25%), a fixed oil and minor constituents like tannin and sugars (Nadkarni, 2010; Chopra *et al* 1956). Zhang *et al*., (2009) isolated eleven compounds and identified as: 5, 7-dihydroxy-2-methylchromone, p-hydroxybenzaldehyde, 3, 5-dimethoxy-4-hydroxy-benzaldehyde, 3, 5-dimethoxy-4-hydroxy-acetophenone, ethyl-2-pyrrolidinone-5(s)-carboxylate, 5-hydroxymethyl-furaldehyde, palmitic acid, succinic acid, glucose, daucosterol, beta-sitosterol. Sesquiterpene lactones such as costunolide and dehydrocostus lactone, are major components of the roots, and have been reported to possess various biological activities such as antimicrobial [17], antifungal [18], antiulcer [19], antiinflammatory [20], anthelmintic [2], Antidiabetic [22], antitumor [23], immuno-stimulant [24] and antihepatotoxic [25].

In present studies phytochemical analysis, total phenolic and flavonoid contents of different solvent extracts of costus root was determined and in addition to their antioxidant potentials were evaluated using various *in vitro* models.

Materials and methods

Chemicals and reagents

2, 2-diphenyl-1-picrylhydrazyl (DPPH·) (supplied by Sigma Aldrich). Ascorbic acid, Folin-Ciocalteu reagent (FCR), gallic acid, sodium carbonate ($NaCO_3$), quercetin, aluminium chloride ($AlCl_3$), potassium acetate, deoxyribose, ferric chloride ($FeCl_3$), ethylene diamine tetraacetic acid (EDTA), H_2O_2, monobasic and dibasic potassium phosphate, thiobarbituric acid (TBA), sodium nitroprusside, sulfanilamide, phosphoric acid (H_3PO_4), naphtyl ethylene diamine dihydrochloride, acetic acid (CH_3COOH), sodium acetate (CH_3COONa), 2,4,6-tripyridyl-s-triazine (TPTZ), ferrous sulfate ($FeSO_4$), phenazine methoxy sulphate (PMS), Nicotinamide adenine dinucleotide (NADH), nitro blue tetrazolium (NBT), HPLC-grade methanol, dichloromethane, and hexane were supplied by S.D. Fine Chemicals Limited (Mumbai).

Preparation of extracts

Costus root powder was extracted with different solvents and antioxidant activity, total phenolic and flavonoid contents were determined. The costus root powder was packed in a thimble and was subjected to Soxhlet extractor using hexane, dichloromethane and methanol as solvents. Refluxing was carried out for 12 hrs. The solution was filtered and the filtrate was concentrated to a minimum volume by distilling off solvent and stored at 4°C.

Phytochemical analysis

The presence of phytochemicals such as flavonoids, phenolics, reducing sugars, saponins, anthraquinones, cardiac glycoside, terpenoids and phlobatannin in the plant were analysed following standard protocols [26]. For anthraquinones, 100 mg of plant extract was boiled with 10 mL of 1% hydrochloric acid and filtered. Filtrate was shaken with 3 mL of benzene and 2 mL of 10% ammonia solution and filtered. Presence of anthraquinones was confirmed by the presence of pink, violet or red colour in the ammonical phase of the solution. Presence of cardiac glycoside was detected when 5 mL (10 mg / ml methanol) of plant extract mixed with 2 mL glacial acetic acid and few drops of ferric chloride. Appearance of a brown ring at the interface of solution after the addition of 1 mL of concentrated sulphuric acid established the presence of cardiac glycosides. Presence of flavonoid was confirmed by the appearance of yellow colour on addition few drops of 1% aluminium chloride solution to 1 mL of plant extract solution.

Appearance of a dark green colour after adding few drops of 5% ferric chloride solution to 0.5g of plant extract dissolved in water indicated the presence of phenolic compounds. When 50 mg of extract was boiled in 1% hydrochloric acid, deposition of red precipitate indicated the presence of phlobatannin. The presence of free reducing sugars was detected by the appearance of a red precipitate in a solution on mixing 2 mL of plant extract (50 mg / ml) and equal volumes of Fehling's solution A and B. Saponins were detected by boiling 50 mg extract with 10 mL distilled water followed by filtration and dilution with distilled water and vigorous shaking to get a stable persistent froth. The frothing was mixed with 2 to 3 drops of olive oil and shaken vigorously. The formation of emulsion indicated the presence of saponins. Presence of terpenoids was confirmed by mixing 5 mL (1 mg / ml) of extract with 2 mL of chloroform and 3 mL of sulphuric acid. A reddish brown colour at the interface confirmed the presence of terpenoids.

Preparation of stock solutions

The stock solution (2 mg / ml) of each compound was prepared by dissolving 20mg compound in 10 ml methanol. The required dilutions of 1.0, 0.5, 0.25, 0.1 and 0.05 mg / ml were subsequently made from the stock solution by adding methanol.

Estimation of total phenolic content

Total phenolic content was determined using Follin-Ciocalteu method [27]. A standard (calibration) curve was obtained with solutions of 1.0, 0.5, 0.25, 0.1 and 0.05 mg / ml of gallic acid. Stock solution (2.0 mg / ml) was made in 10 ml of methanol and then further dilutions were made. In 100 µl of each concentration of standard solution, 100 µl of Folin-Ciocalteu reagent and 2 ml of 2% sodium carbonate (Na_2CO_3) were mixed. The resulting mixture was allowed to stand at room temperature for 30 min and the absorbance was measured at 743 nm using UV-Visible Spectrophotometer against a blank prepared similarly but containing distilled water instead of standard solution of gallic acid. A standard curve was obtained by plotting absorbance against amount of gallic acid.

Estimation of flavonoids content

For estimation of total flavonoids [28] in extracts of costus roots added 0.1 ml of 10% aluminium chloride and 0.1 ml of potassium acetate (1M) in 0.5 ml of extract. In this mixture 4.3 ml of 80% methanol was added to make 5 ml volume. The absorbance was measured at 415 nm using UV-VIS double beam Spectrophotometer against a blank containing respective solvent without extracts. The amount of total flavonoids present in the extracts was calculated from the standard curve and results were expressed as milligrams of quercetin equivalent per gram (mg QE g^{-1}).

Antioxidant activity

The antioxidant potential was evaluated using different *in vitro* models involving 2, 2-diphenyl-1-picrylhydrazyl (DPPH•), hydroxyl (OH•), nitric oxide (NO•), and ferric reducing antioxidant power assay (FRAP).

DPPH radical scavenging method

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging was determined by the method [29]. A 0.1 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 1ml of various concentrations (0.05-0.8 mg / ml) of sample dissolved in methanol to be tested. The reaction mixture was left in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm using spectrophotometer. Ascorbic acid was used as a reference material. All tests were performed in triplicate. The percentage inhibition was calculated as follows:

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Hydroxyl radical scavenging method

Hydroxyl radical scavenging activity was determined by deoxyribose degradation method [30]. The reaction mixture consisted of deoxyribose (2.8 mM), ferric chloride (0.1 mM), ethlene diamine tetraacetic acid (0.1 mM), hydrogen peroxide (1 mM), ascorbic acid (0.1 mM), phosphate buffer (20 mM, pH 7.4) and various concentrations of selected components in a final volume of 1ml. The reaction mixture was incubated for 1hr at 30 ºC. For this, 3ml thiobarbituric acid (0.67%) was added to each test tube and kept for 1hr in boiling water. The absorbance of the chromophore formed was read at 532 nm against blank prepared in similar way. Control was also run parallel, in which no compound was added. The percentage inhibition was determined by comparing the absorbance values of tested components and control using above formula.

Nitric oxide scavenging method

Nitric oxide radical scavenging was determined using method given by Green *et al* [31]. In a test tube 0.5 ml volume of various concentrations(0.05-1g / ml) of extracts was mixed with 0.5 ml of sodium nitroprusside (10 mM) and 0.5 ml phosphate buffered saline (PBS) and incubated at 30ºC for 2.5 hrs. Control without test compounds and blank without sodium nitroprusside but with equivalent amount of buffer were kept in identical manner. After incubation, 1 ml of of Gries reagent (1% sulfanilamide, 2% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added. The absorbance of chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling to napthyl ethylene diamine was read at 548 nm against blank after making the final volume of 3 ml with distilled water. The percentage inhibition of nitric oxide generation was measured by comparing the absorbance value of tested components and control using above formula.

Ferric reducing antioxidant power (FRAP assay)

Ferric reducing antioxidant power was determined by the method of Benzie and Strain [32]. The stock solutions included 300 mM acetate buffer (3.1 g hydrated sodium acetate and 16 ml acetic acid) at pH 3.6, 10 mM 2, 4, 6-tripyridyl-s-triazine solution in 40 mM hydrochloric acid and 20 mM hydrated ferric chloride solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml 2, 4, 6-tripyridyl-s-triazine and 2.5 ml hydrated ferric chloride. The temperature of the solution was raised to 37°C before use. Tested compounds (0.2 ml) were allowed to react with 2.8 ml of the FRAP solution for 30 min in the dark condition. Readings of the coloured product (ferrous tripyridyltriazine complex) were taken at 593 nm. Results were expressed in terms of µg Fe (II)/g dry mass.

Superoxide radical scavenging method

Measurement of superoxide anion scavenging activity of resveratrol was based on the method described by Liu *et al* [33]. Superoxide anions were generated in a non-enzymatic phenazine methoxy sulfate-nicotinamide adenine dinucleotide (PMS-NADH) system by oxidation of NADH and assayed by reduction of nitro blue tetrazolium (NBT). In this experiment, the superoxide anion was generated in 1 ml of phosphate buffer (pH 7.4) containing 1 ml of NBT (50 µM) solution, 1 ml of NADH (78 µM) solution and 1ml of different concentrations of all tested extracts of costus. The reaction was started by adding 1 ml of PMS solution (60 µM) to the mixture. The reaction mixture was incubated at 25°C for 5 min and absorbance at 560 nm was recorded against blank samples in a spectrophotometer.

Results and Discussion

Phytochemical analysis

Presence of different phytochemicals was detected qualitatively in all the extracts and results are tabulated in Maximum number of phytochemicals were detected in methanol extract.

Table 1: Phytochemical analysis of costus roots extracts

Chemical constituent	Methanol extract	DCM extract	Hexane extract
Flavonoids	+	+	+
Phenolics	+	+	+
Reducing sugar	+	-	-
Saponins	+	+	+
Anthraquinones	-	-	-
Cardiac glycoside	-	-	-
Terpenoids	+	+	+
Phlobatannin	+	-	-

(+) indicated the presence and **(–)** indicated absence

Total phenolic and flavonoid content

The total phenolic content (TPC) in extracts of costus roots are presented in Table 2. Results were expressed as mg GAE/g of extract using the gallic acid standard curve. The amount of phenols varied from 12.34 to 75.02 mg GAE/g in the test components. In the methanol extract of costus a high content of total phenols (75.02mg GAE/g of extract) was obtained. Dichloromethane extract contained moderate level of phenolic content (52.34 mg GAE/g, respectively), while hexane extract contained the lower level of phenolic content (12.32 mg GAE/g). It was considered that the antioxidant activity of phenolic compounds was due to their redox properties, which played an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [34].

The total flavonoid content (TPC) in extracts of costus roots are presented in Table 2. Results were expressed as mg QE/g of extract using the quercetin standard curve. The amount of flavonoids varied from 16.2 to 67.60 mg QE/g in the tested components. Methanol extract showed high content of total flavonoids (67.60 mg QE/g) than other extracts. Dichloromethane extract contain moderate level of flavonoid

content (45.85 QE/g, respectively), while lower level of phenolic content (16.20 mg QE/g) was found in hexane extract. It is considered that the antioxidant activity of flavonoid compounds may be due to their scavenging or chelating process [35].

Table 2: Total phenolic content and total flavonoid content of various extracts

Components	Total phenols mg GAE/g of extract	Total flavonoids mg QE/g of extract
Methanol extract	75.02±0.42	67.60±1.97
Dichloromethaneextract	52.34±0.56	45.85±1.62
Hexane extract	12.45±0.26	16.20±0.28

Results were expressed as mean± SD

4.3 DPPH free radical scavenging

Different costus root extracts were evaluated for their antioxidant potential by *in vitro* 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method at different concentration levels *viz*. 1.0, 0.5, 0.25, 0.1 and 0.05 mg / ml (Table 3). Ascorbic acid was used at standard. Methanol showed 52.56% scavenging at 0.25 mg / ml whereas ascorbic acid at the same concentration exhibited 82.23% scavenging. It was observed that methanol showed highest activity among the tested extracts while ascorbic acid used as standard was more effective than extracts. DCM extract activity showed a linear increase with concentration and showed 65.32% scavenging at 0.5 mg / ml. Activity of hexane extract and dichloromethane extracts was comparable. Hexane extract was least active among all extracts tested. It was observed that DPPH free radical scavenging activity of all the tested components increased with increase in concentration. Good antioxidant activity of the tested extracts was due to their high proton donating ability. All the extracts showed good antioxidant activity but less than that of ascorbic acid.

Table 3: DPPH free radical scavenging potential (%) of various extracts of costus roots

Extracts	Concentrations(mg / ml)				
	0.05	0.1	0.25	0.5	1.0
Methanol extract	11.75 ± 0.11	35.21 ± 0.11	52.56 ±0.11	74.23±0.35	94.54±0.23
Dichloromethane extract	9.05 ±0.22	25.05 ±0.22	43.26±0.23	65.32±0.22	86.41±0.22
Hexane extract	5.55±0.75	23.26±0.23	41.87±0.23	64.51±0.22	89.70±0.07
Ascorbic acid(Standard)	38.23±1.06	63.13±1.28	82.24±1.99	95.16±0.87	100 ± 0.00

Results were expressed as mean± SD.

Hydroxyl radical scavenging

Hydroxyl radical scavenging activity of methanol, dichloromethane and hexane extracts of costus roots varied widely and increased with increase in concentration levels (Table 4). Methanol extract of costus roots showed more than 50% scavenging activity i.e. 58.43% inhibition of at 0.25 mg/ml. Activity of dichloromethane and hexane extract was comparable at high concentrations but hexane was least active amongst all the tested extracts. At concentration to 0.5 mg/ml activity of DCM extract was 55.35 % and that of hexane extract was 52.18%. All the tested extracts showed a positive correlation between concentration and activity i.e. activity increase with increased in concentration.

Table 4: Hydroxyl radical scavenging potential (%) of various extracts of costus roots

Extracts	Concentrations (mg / ml)				
	0.05	0.1	0.25	0.5	1.0
Methanol extract	19.87± 2.21	31.87± 1.12	58.43± 1.08	75.62± 2.79	89.65± 0.52
Dichloromethane extract	11.62 ± 2.16	25.62±2.56	40.31± 2.26	55.1± 3.21	73.43± 1.55
Hexane extract	9.31± 1.12	18.31± 3.22	33.43± 0.71	52.18± 1.29	69.22± 2.52
Ascorbic acid(Standard)	32.89±1.80	56.47±1.25	67.50±1.90	80.35±1.65	98.83±2.05

Nitric oxide radical scavenging

Nitric oxide is a very unstable species and reacts with oxygen molecule to produce stable nitrate and nitrite which can be estimated using Griess reagent. In the presence of a scavenging test compound, the amount of nitrous acid decreased which can be measured at 546 nm [36]. Nitric oxide radical scavenging activity of methanol, dichloromethane and hexane extracts of costus root varied widely and increased with increase in concentration level (Table 5). Methanol extract of costus root showed 55.58% inhibition at concentration 0.25 mg/ml. Dichloromethane extract showed maximum inhibition of 73.23% at 1.0 mg/ml while hexane showed 72.08% inhibition at same concentration thus activities of both extracts were comparable at highest concentration tested. Hexane extract was least effective among all extracts tested. All the extracts showed good antioxidant activity. However, methanol extract exhibited higher antioxidant activity which was more than standard ascorbic acid. Nitric oxide radical scavenging activity was correlated to the presence of phenolic compounds[37].

Table 5: Nitric oxide free radical scavenging potential (%) of various extracts of costus roots

Extracts	Concentrations(mg / ml)				
	0.05	0.1	0.25	0.5	1.0
Methanol Extract	21.62±0.51	33.62±0.51	55.58±0.23	79.63±0.04	89.63±0.04
Dichloromethane extract	13.67±0.04	23.67±0.04	40.35±0.14	59.28±0.28	73.23±0.46
Hexane Extract	8.92±0.79	14.92±0.79	33.80±0.14	52.04±0.23	72.17±0.46
Ascorbic acid(Standard)	31.50±0.96	58.42±1.81	75.54±0.53	89.49±1.22	99.89±0.93

Results were expressed as mean± SD

Ferric reducing antioxidant power

Ferric reducing antioxidant power assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ), forming an intense blue Fe^{2+}-TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH 3.6). The decrease in absorbance is proportional to the antioxidant content (Politeo *et al* 2011). The reducing ability of all the tested components was expressed in terms µmol Fe (II)/ml of the concentration of the extract (Table 9). The antioxidant potential of the tested extracts were estimated from their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). The reducing abilities of the tested components compared with ferrous sulphate standard curve. The reducing power (absorbance at 593 nm) of the tested components was concentration dependent which increased with increase in concentration. Frap value of methanol extract was more than all other extract. Hexane have minimum FRAP value among all extracts at all the concentrations tested. The FRAP values of different extracts at various tested concentration was tabulated in Table 6.

Table 6: FRAP values (Fe^{2+} µmol / ml) of of various extracts of costus roots

Extracts	Concentrations(mg / ml)				
	0.05	0.1	0.25	0.5	1.0
Methanol Extract	79.85 ± 3.21	168.1 ± 2.69	285.15 ±4.54	718 ±6.44	954.45 ±5.64
Dichloromethane extract	74.63 ±3.21	162.63 ±2.50	267.0 ±5.43	558.12 ±5.51	848.64 ±7.41
Hexane Extract	27.27±3.21	86.27±1.21	190.12±4.21	335.31±4.61	751.16±10.21

Results were expressed as mean± SD.

Superoxide radical scavenging activity

Superoxide radical scavenging activity of methanol, dichloromethane and hexane extracts of costus seeds varied widely and increased with increase in concentration levels (Table 7). Methanol extracts of costus seeds showed maximum inhibition of 81.82% at 1.0 mg/ml. Dichloromethane extract showed maximum inhibition of 66.85% at 1.0 mg / ml. Hexane extract showed maximum inhibition of 49.63 % at 1.0 mg/ml. All the extracts showed good antioxidant activity. Methanol was more effective among all the tested components although all extracts were less effective as compare to ascorbic acid used as standard. Hexane extract was least active as it showed less than 50% inhibition if at maximum tested concentration 1.0 mg/ml.

Table 7: Superoxide free radical scavenging potential (%) of various extracts of costus roots

Extracts	Concentrations (mg / ml)				
	0.05	0.1	0.25	0.5	1.0
Methanol extract	15.23±0.67	31.14±1.31	41.08±1.26	62.14±1.42	81.82±2.04
Dichloromethane extract	9.25±1.10	23.54±1.14	32.56±2.21	51.23±1.12	66.85±2.11
Hexane extract	6.21±2.31	14.67±1.21	25.23±0.31	39.81±2.23	49.63±1.31
Ascorbic acid(Standard)	29.10±0.47	46.27±0.74	53.09±0.53	68.90±0.46	88.19±0.52

Results were expressed as mean± SD.

Conclusions

Due to harmful effect of synthetic antioxidants plant-derived antioxidants remain an important resource to combat various health problems. In present study it was found that costus root extracts are effective as antioxidants. Methanol extract contained maximum number of phytochemicals and alo contained highest phenolic and flavonoid content among all the extracts tested. Methanol extract exhibited more inhibition than all the tested extracts. It is known that phenolic compounds show antioxidant activity thus antioxidant potential of methanolic extract of costus root can be related to the high phenolic and flavonoid content present.

The overall order of antioxidant potential of costus roots was as follows:

Ascorbic Acid > Methanol extract >Dichloromethane extract > Hexane extract

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