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Studies on Antioxidant and Antibacterial Activity of *Cissampelos pareira* (L.)

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Abstract

The present study was aimed to investigate the *in vitro* callus induction, phytochemical analysis, antioxidant and antibacterial activity of *C. pareira*. The sterilized explants were inoculated on MS medium supplemented with different concentration and combinations of auxins and cytokinins for callus induction. The methanolic extracts of leaf, stem, root and callus were used for phytochemical and antioxidant assays using standard methods. The antibacterial activity was analyzed by agar well diffusion method against different bacterial strains. The significant percentage of callus induction was observed on MS medium fortified with 2, 4-D, NAA and in combination of NAA + KIN showed the significant percentage of callus induction from leaf and stem explants. The maximum content of total flavonoids and alkaloids were recorded in root. Whereas, higher total tannins and phenolic content were observed in stem and callus respectively. The methanolic stem extracts of *C. pareira* showed significant antioxidant activity followed by leaf and callus extract. The maximum zone of inhibition was observed in root and stem against *K. pneumoniae* and *P. aeruginosa* respectively. In conclusion, effective protocol for callus induction was developed and further studies have to be carried out to isolate and characterize the bioactive compounds from this important medicinal plant.

Keywords: *C. pareira*, Menispermaceae, Auxins, Phytochemicals, Antioxidants.

1. Introduction

Medicinal plants have been discovered and used in traditional medicine practices since prehistoric times. Today a significant number of drugs have been developed from medicinal plants. The herbal medicines are considered to have great importance among different rural or indigenous communities in many developing countries. Traditional medicine system includes the knowledge, skills and practices based on the theories, beliefs and experiences of the folks communities to maintain their health problems. The indigenous communities have their own traditional medicine system with different medicinal plants and traditional therapies for incurable diseases (Gosh, 2003).

Since the ancient times medicinal plants are lauded for their pharmacological properties due to the presence of secondary metabolites such as alkaloids, flavanoids, glycosides, tannins, steroids, etc. Some of these secondary metabolites are important sources of natural antioxidants which reduced the risk and progression of certain diseases.

Cissampelos pareira (*C. pareira*) was first described from Latin America, occurs throughout the tropics. *C. pareira* is a dioecious plant, sub-erect climbing herb usually known as velvet leaf belongs to the family Menispermaceae (Vaidya, 1998). The plant is frequently used for treatment of various ailments like cough, abdominal pain, kidney stones, asthma, arthritis, diarrhea, dysentery, kidney infection and fever as stated by Ayurvedic Pharmacopedia of India (Gessler et al., 1994; Caceres et al., 1987). *C. pareira* contains many secondary metabolites such as alkaloids (bisbenzylisoquinoline, hayatine, hayatidine, berberine, cissampareine, dicentrine, insularine, cycleanine, curine and isomerubrine), flavanoids, tannins, volatile oils and glycosides etc. The plant possess many pharmacological activities like anti-inflammatory, analgesic, antipyretic, immunomodulatory, antivenom, memory-enhancing, anti-diarrhoeal, antidiabetic, hepatoprotective, muscle relaxant, anti-urolithic, cardiovascular, antioxidant, anticancer,

antiulcer, antiparasitic, antimalarial, antimicrobial, anti-diuretic and anti-dengue (Kamal et al., 2017).

Based on the above facts, the present study was aimed to investigate the antioxidant, antibacterial and secondary metabolites from methanolic extracts of leaf, stem, root and callus.

2. Materials and Methods

2.1. Collection, sterilization of explants and callus induction

Plants were collected in and around the Jnanabharathi campus and maintained at the Department of Botany in the greenhouse condition. The leaf and stem explants were surface sterilized with 0.1 % bavistin for 20 min, followed by liquid detergent (1-2 drops of teepol) and kept under running tap water to remove the traces of detergent followed by three to four rinses with distilled water (d.w). Finally, the explants were sterilized with 0.1 % mercuric chloride for 3 min and rinsed 3-4 times with double distilled water. The sterilized explants were transferred into the culture bottles.

MS medium containing 3 % sucrose and 0.8 % agar (Plant tissue culture grade, Himedia) supplemented with different concentration (0.5 - 4 mg/L) and combination of auxins (2, 4-D, NAA, IAA and IBA) and cytokinins (BAP, KIN) for callus induction. The pH of the media was adjusted to 5.6 - 5.8, then autoclaved at 121 $^{\circ}$C for 20 min. The cultures were incubated at 25 ± 2 $^{\circ}$C under 16 h photoperiod with light intensity (3000-4000 Lux) provided by white florescent bulbs.

2.2. Quantitative analysis

2.2.1. Preparation of plant extracts

The dried callus, leaf, stem and root was powdered with the help of mortar and pestle. The samples were extracted with 90 % methanol by using soxhlet apparatus for 12 -14 h. The extracts were kept for evaporation till dryness and were stored in the refrigerator until further use.

Estimation of total flavanoids

The total flavanoids content in the methanolic plant extract was determined by aluminum chloride method (Rajendran et al., 2014). 0.5 ml of extract, 2.5 ml of 95 % ethanol, 0.2 ml of 10 % aluminum chloride, 0.2 ml of 1M potassium acetate was added and make up the final volume to 9 ml with d.w. The mixture was kept at room temperature (RT) for 30 min. The absorbance of the reaction mixture was measured at 415 nm using UV-spectrophotometer. The final absorbance of each sample was compared with a standard curve. The total flavanoids content was expressed in µg of quercetin per mg of extract.

2.2.2. Estimation of total Phenolics

The total phenolics content of methanolic plant extract was determined using Folin-Ciocalteu method (Singleton et al., 1999). 0.5 ml of plant extract was mixed with 0.2 ml of Folin- Ciocalteu reagent and the mixture was allowed to stand at room temperature for 5 min. 2 ml of sodium carbonate (7.0 %) was added and the reaction mixture was made up to 5 ml with sterile d.w. The reaction was allowed to stand for 90 min in dark. Then the absorbance of the blue color developed by the mixture was measured at 725 nm using UV-spectrophotometer against the blank. The final absorbance of each sample was compared with a standard. The total phenolic content of plant extracts was expressed as µg of gallic acid per mg of extract.

2.2.3. Estimation of total Tannins

The total tannins in the plant extracts were estimated using Folin-Denis method (Polshettiwar et al., 2007). 0.5 ml of the sample, 0.5 ml of Folin-Denis Reagent, then 1 ml of sodium carbonate (7.0 %) was added and the reaction mixture was made up to 10 ml with sterile d.w. Then the absorbance of blue color developed by the reaction mixture was measured at 700 nm using UV-spectrophotometer against blank. The absorbance of sample was compared with a standard curve. The total tannins were expressed as µg of tannic acid per mg of extract.

2.2.4. Estimation of total alkaloids

The total alkaloid content in methanolic plant extracts, were determined by 1, 10-phenantroline method (Singh et al., 2004), using Colchicine as standard. 0.5 ml of extract, 1 ml of 0.025 M Ferric chloride in 0.5 M hydrochloric acid and 1 ml of 0.05 M of 1, 10-phenantroline in ethanol was added. The mixture was incubated for 30 min in hot water bath and maintained at 70 °C. The absorbance of red colored complex was measured at 510nm against reagent blank. The total alkaloid content was calculated with the help of standard curve. The total alkaloid was expressed as µg of colchicine per mg of extracts.

2.3. Antioxidant activity

2.3.1. DPPH radical scavenging activity

The antioxidant activity of the methanolic extracts was determined by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay (Mensor et al., 2001). The DPPH solution was prepared by dissolving 29 mg of DPPH in 100ml of 95 % methanol. Different concentration of plant extracts (100, 200, 300, 400, 500 and 600 µg/ml), were made up to 1 ml using methanol. 1 ml of sample was added to 1 ml of DPPH solution and the reaction mixture was allowed to stand in dark for 30 min at RT. The absorbance of the reaction mixture was measured at 415 nm. The percentage of inhibition was calculated as using following formula,

$$\% \text{ of inhibition} = \frac{(A^o - A^1)}{A^o} \times 100$$

Where A^o is the absorbance of the control and A^1 is the absorbance of the sample.

The DPPH radical scavenging activity of the sample was expressed as IC_{50} value.

2.3.2. ABTS scavenging activity

The ABTS radical scavenging activity of the plant samples were determined according to the method of Shirwaikar et al. (2006). The ABTS radical cation was produced by reacting 5 ml of 7 mM ABTS solution with 5 ml of 2.45 mM ammonium persulphate, stored in dark for 16 h at RT. To 1 ml of plant extracts with different concentration (20 – 350 µg/ml), was added with 3 ml of diluted ABTS reagent. The absorbance of the test sample was read at 725 nm. The percentage of inhibition was calculated using the formula,

$$\% \text{ of inhibition} = \frac{(A^o - A^1)}{A^o} \times 100$$

Where A^o is the absorbance of the control and A^1 is the absorbance of the sample.

The ABTS scavenging activity of the plant sample was expressed as IC_{50} value.

2.3.3. Reducing power assay

The reducing power assay was determined according to the procedure of Oyaizu (1986). Various concentration of plant extract (50 - 300 µg/ml), 2.5 ml of 0.2 M phosphate buffer and 2.5 ml of 1 % potassium ferricyanide was added. The reaction mixture was incubated for 20 min at 50 °C and then cooled. To this mixture add 2.5 ml of 10 % trichloroacetic acid and centrifuged at 3000 rpm for 10 min. To 2.5 ml of the supernatant was added with 2.5 ml of d.w and freshly prepared 0.5 ml of 0.1 % ferric chloride. The absorbance was read at 700 nm.

2.3.4. Total antioxidant capacity

The total antioxidant capacity was evaluated by the method described by Prieto et al. (1999). The Phosphomolybdenum reagent contained 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. 0.5 ml of the plant sample, 3 ml of phosphomolybdenum reagent was added. The reaction mixture was incubated at 95 °C for 90 min. and cooled. The absorbance of the green phosphomolybdenum complex was measured at 695 nm. The reducing capacities of the analyzed extracts were expressed as µg of ascorbic acid per mg of extract.

2.4. Antibacterial assay

The antibacterial screening was carried out using the agar well diffusion method described by Lino and Deogracious (2006) with slight modifications. The bacterial strains were obtained from Department of Microbiology, Bangalore University, Bengaluru. Each tested bacterial strains (*Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, and*

Klebsiella pneumoniae) were inoculated into nutrient broth and incubated at 37 °C for 18 h. Each of the cultures were then adjusted to 0.5 McFarland turbidity ($1x10^8$ CFU/ml.) and subjected to nutrient agar plates. The bacterial cultures were applied evenly on the NA plates. The wells were made using a sterile cork borer (8 mm in diameter). The ofloxacin was used as positive and the DMSO was used as negative control.

The culture plates were allowed to stand on the working bench for 30 min for pre diffusion and were then incubated at 37 °C for 24 h. After the incubation, antibacterial activity was measuring by the zones of inhibition in mm against the test organisms. The experiment was repeated in triplicates.

2.5. Statistical Analysis

The results were expressed as mean ± standard deviation (S.D). Data were analyzed by one way analysis of variance followed by Duncan's multiple range tests using SPSS software. Probability values $P < 0.05$ were considered significant.

3. Results and Discussion:

3.1. Callus induction

Callus induction from leaf and stem explants of *C. pareira* was observed in MS medium supplemented with different concentration and combinations of auxins and cytokinins. The initiation of callus was observed after 2-3 weeks of culture. The profused and friable callus was observed in 2, 4-D (3 mg/L) and NAA (4 mg/L). The maximum callus induction was observed in the combination of 0.5 mg/L of BAP + 1.5 mg/L of IAA and 2 mg/L of NAA + 0.5 mg/L of KIN (Fig. 1 A to D). Our results were in concordance with Thangavel et al. (2011) where, maximum percentage of callus induction was observed in *Centella asiatica* from leaf explants.

3.2. Quantitative analysis

In the present study methanolic extracts of leaf, stem, root and callus were carried out for the quantification of phytochemicals (Table. 1). Methanol solvent is polar in nature, most of the bioactive compounds are soluble in methanol. The methanolic extract of stem showed maximum flavonoids and tannin content (50.29±4.16 µg/ml and 42.33±1.28 µg/ml) compared to other extracts. Highest concentration of alkaloid and phenolic content was recorded in methanolic extract of root and callus (518.51±0.98 µg/ml and 65.27±0.57 µg/ml) respectively. The results obtained were similar to the observation made by Manasa et al. (2017**)** in *Mussaenda frondosa*, Anupa et al. (2017) and Zahoor et al. (2016) were also observed the maximum content of phenolics and flavanoids in ethanolic extract of *C. pareira* leaf.

3.3. Antioxidant activities

The antioxidant assays were carried out from the methanolic extracts of leaf, stem, root and callus (Table. 2). The IC_{50} value of DPPH (325.62 µg/ml) and ABTS (55.17 µg/ml) radical activity was found to be maximum in methanolic extract of stem compared to other extracts. Whereas, the reducing power and total antioxidant capacity was noticed in methanolic extract of stem and callus respectively. Similar results were reported by Santhosh and Asha (2013), where methanolic extracts of *Coscinium fenestratum* stem showed more free radical antioxidant activity compared to leaf extracts. Seetharaman et al. (2018) were also reported that methanolic extracts of *C. pareira* leaf exhibited highest free radical scavenging activity. Whereas, the DPPH radical scavenging activity was found to be maximum in methanolic extracts of *Cocculus hirsutus* stem followed by leaf and callus (Mukesh et al., 2014).

3.4. Antibacterial activity

Antibacterial activity of methanolic extracts in leaf, stem, root and callus were done by agar well diffusion method against different bacterial strains. Among the different plant extracts, root and stem showed the maximum activity against *K. pneumoniae* (11.16±1.04 mm) and *P. aeruginosa* (9.5±1.0 mm) (Fig. 2 and 3 A-D). Leaf extracts recorded highest activity against *E. coli* and *P. aeruginosa* compared to other extracts. Methanolic extract of callus showed significant activity against *B. subtilis, E. coli* and *S. aureus* when compared to other strains. The results were concordance with the reports of Kumar et al. (2012), where methanolic root and bark extracts of *C. pareira* and *Ficus racemosa* showed the maximum antibacterial activity against *P. aeruginosa, S. aureus* and *B. subtilis*. Njeru et al. (2014) also reported that methanolic root extract of

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C. pareira showed significant zone of inhibition against *S. aureus, K. pneumoniae and E. coli* compared to other strains.

4. Conclusion

The present investigation reveals an effective protocol for callus induction from leaf and stem explants of *C. pareira*. The methanolic extract of this plant showed a rich source of phyto constituents like alkaloids, flavanoids, tannins, phenols etc. The *C. pareira* contains an important source of natural antioxidants, which helps to prevent the oxidative stress. In our study, the plant showed potential antibacterial activity against different bacterial strains. Further studies has to be carried out to elucidate and enhance the bio active compounds from this medicinally important plant.

Table. 1: Estimation of Total Flavanoids, Phenols, tannins and alkaloids of methanolic extracts of *C. pareira.*

Plant samples	Total flavanoids µg/mg	Total Phenols µg/mg	Total tannins µg/mg	Total alkaloids µg/mg
Leaf	44.91±0.64	56.76±0.81	12.98 ± 0.56	88.22 ± 1.64
Stem	50.29±4.16	49.85±0.83	42.33 ± 1.28	305.08 ± 2.12
Root	46.06±1.94	44.25±0.90	41.79±0.636	518.51 ± 0.98
Callus	44.37±0.95	65.27±0.57	17.99 ± 0.17	248.58 ± 1.78

Values in the represent the Mean ± SD of triplicate determination.

Table. 2: Antioxidant activities of methanolic extracts of *C. pareira*.

Plant Samples	DPPH IC_{50} (µg/ml)	ABTS IC_{50} (µg/ml)	Reducing power µg/mg	Total antioxidant capacity µg/mg
Leaf	559.39±07.92	096.06±13.39	078.26±0.31	34.73±0.12
Stem	325.62±17.07	055.17±04.00	101.18±0.41	45.73±0.37
Root	571.75±12.53	122.34±03.85	086.23±1.23	37.35±0.44
Callus	537.31±07.30	064.87±17.21	062.83±0.99	51.79±0.78

Values in the represent the Mean ± SD of triplicates determination.

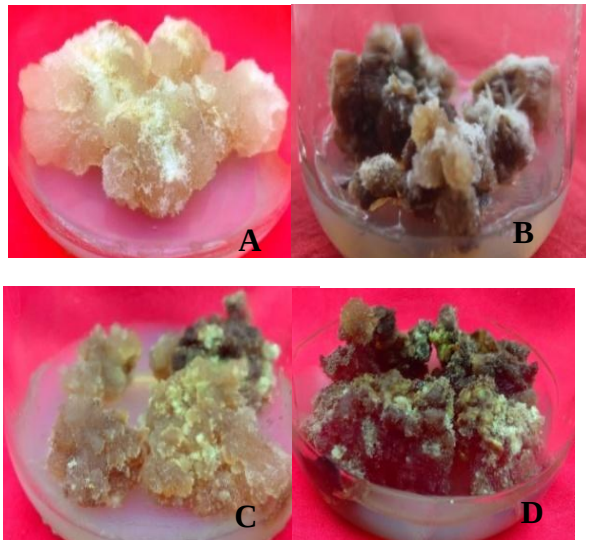

Fig. 1: Effect of auxin and cytokinin on callus induction from leaf and stem explants: **A**- MS + 2, 4-D (4mg/L), **B**- MS + NAA (2mg/L), **C**- MS + BAP (0.5mg/L) + IAA (2 mg/L), **D**- MS + NAA (2mg/L) + KIN (0.5mg/L).

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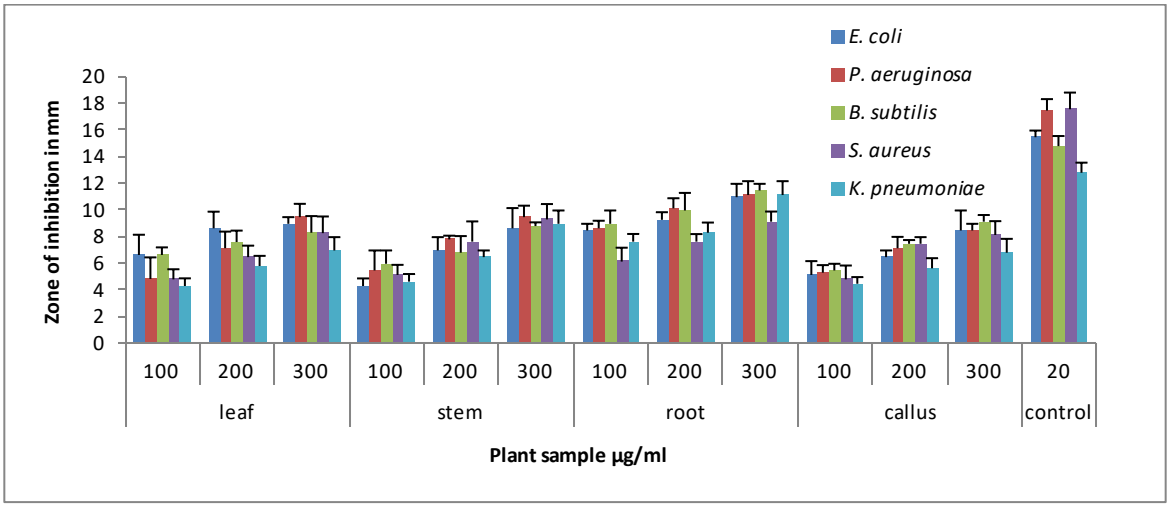

Fig. 2: Antibacterial activity of methanolic extracts of *C. pareira* against bacterial strains.

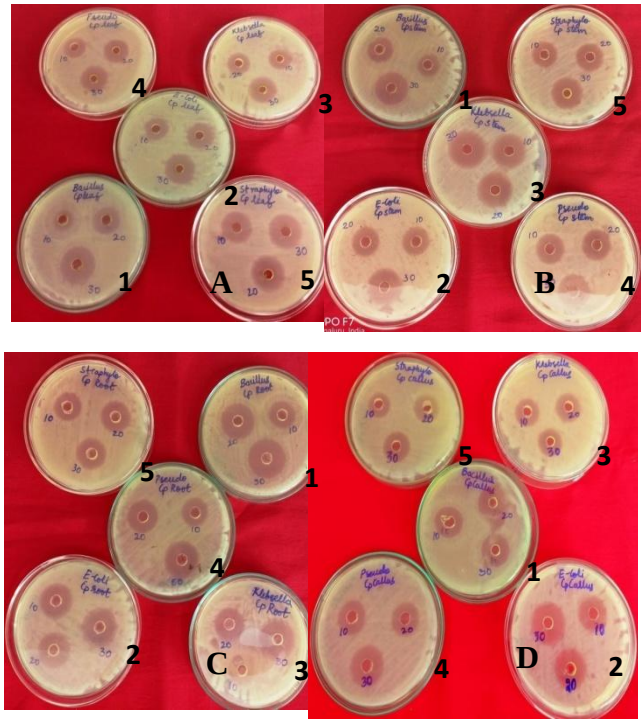

Fig. 3: Zone of inhibition from different bacterial strains; **A**-Leaf, **B**-stem, **C**-root and **D**-callus.

1 - *B. subtilis*, **2** - *E. coli*, **3** - *K. pneumoniae*, **4** - *P. aeruginosa*, **5** - *S. aureus*.

Reference

1. Anupa MP, Vasantha KB and Thavasimuthu C, *In vitro* antioxidant activity of leaf extracts of *Cissampelos pareira* L var. Hirsuta (Buch.-Ham. Ex DC.) Forman, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 8(2): 947-955, (2017).
2. Caceres A, Giron LM and Martínez AM, Diuretic activity of plants used for the treatment of urinary ailments in Guatemala, Journal Ethno pharmacology, 19: 233-245, (1987).
3. Gessler MC, Nkunya MH, Mwasumbi LB, Heinrich M and Tanner M, Screening Tanzanian medicinal plants for antimalarial activity, Acta Trop, 56: 65-77, (1994).
4. Gosh A, Herbal folk remedies of Bantura and Medinipur districts, West Bengal (India), Indian Journal of Traditional Knowledge, 2: 393-396, (2003).
5. Kamal S, Shaiba SQ, Jeetendra KG, Neeraj U and Nagendra SC, Ethnomedicinal uses, Phytochemistry and pharmacology of *Cissampelos pareira:* A Review, Medicinal plants and its therapeutic uses, OMICS Group eBooks. 1-19, (2017).
6. Kumar CP, Chandra DS and Kumar DS, Antimicrobial activity of *Ficus racemosa* and *Cissampelos pareira* L. Var Hirsuta (DC) Forman., Against *E-coli, P. aeruginosa, S. aureus* and *B. subtilis*, International Research Journal of Pharmacy, 3(2): 151-153, (2012).
7. Lino A and Deogracious O, The *In vitro* antibacterial activity of *Annona senegalensis, Securidaccalongi pendiculata* and *Steganotaenia araliacea*, African Journal of Health Sciences, 6: 31-35, (2006).
8. Manasa DJ, Chandrashekar KR and Bagya N, Rapid *In vitro* callogenesis and phytochemical screening of leaf, stem and leaf callus of *Mussaenda frondosa* Linn: A medicinal plant, Asian Journal of Pharmaceutical and Clinical Research, 10(6): 81-86, (2017).
9. Mensor LI, Menezes FS, Leitao GG, Reis AS, Dos Santos T, Coube CS and Leitao SG, Screening of Brazilian plants extracts for antioxidants activity by the use of DPPH free radical method, Phytother. Res, 15: 127-130. (2001).
10. Mukesh KM, Neelam S and Vidya P, Evaluation of *in vitro* antioxidant potential of methanolic extract of *Cocculus hirsutus* (L). Diels, International Journal of Pharmacy, 4(1): 204-207, (2014).
11. Njeru SN, Musa R, Mwaniki SN and Oduor PL, Screening of antimicrobial activity of *Cissampelos pareira* L. methanol root extract, European Journal of Medicinal plants, 4(1): 45-51, (2014).
12. Oyaizu M, Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine, Japanese Journal of Nutrition, 44: 307-315, (1986).
13. Polshettiwar SA and Ganjiwale RO, Spectrophotometric estimation of total tannins in some Ayurvedic eye drops, Ind J Pharmacy Sci, 69(4): 574-576, (2007).
14. Prieto P, Pineda M and Aguilar M, Spectrophotometric quantitative of antioxidant capacity through the formation of a Phosphomolybdenum complex: Specific application to the determination of vitamin E, Anal. Biochem, 269: 337-341, (1999).
15. Rajendran C, Christopher FC, Ramu N, Pazhamalai V, Mohankumar N and Ethiraj S, Studies on phytochemical screening, antioxidant activity and extraction active compound (2-Hydroxy 4-Methoxy Benzaldehyde) from rhizome extract of *Decalepis hamiltonii* Wight and Arn, International Journal of Current Biotechnology, 2: 31-39, (2014).
16. Santhosh WG and Asha A, Evaluation of antimicrobial and antioxidant activity of stem and leaf extracts of *Coscinium fenestratum*, Asian Journal of Pharmaceutical and Clinical Research, 6(1): 218-221, (2013).
17. Seetharaman S, Indra V, Durairasu M and Sangeetha B, Phytochemical profiling antibacterial and antioxidant potential of *Cissampelos pareira* L. leave extracts, International Journal of Chemistry Studies, 2(2): 88-90, (2018).
18. Shirwaikar A, Shirwaikar A, Rajendran K and Punitha ISR, *In vitro* antioxidant studies on the benzyl tetra Isoquinoline alkaloid berberine, Biol. Pharm. Bull. 29: 1906-1910, (2006).
19. Singh DK, Srivastava B and Sahu A, Spectrophotometric determination of

Rauwolfia alkaloids: Estimation of reserpine in pharmaceuticals, Analytical Science, 20(3): 571-573, (2004).

20. Singleton VL, Orthofer R and Lamuela - Raventos RM, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, Methods Enzymol, 299: 152-178, (1999).

21. Thangavel A, Muniappan A, Yesudasan JKP and Thangavel S, Phytochemical screening and antibacterial activity of leaf and callus extracts of *Centella asiatica*, Bangladesh J Pharmacol, 6: 55-60, (2011).

22. Vaidya GB, Adarsha Nighantu, Chaukhambha Bharti Academy Publications, 1(2): 153, (1998).

23. Zahoor MG, Sambhamurthy C, Mohd Yasin B, Radheshyam M, Insaf AQ and Irfan AG, Antioxidant and enzyme inhibitory activities of *Cissampelos pareira* L. leaf extracts, Annals of Phytomedicines, 5(1): 91-98, (2016).