INNOVARE JOURNAL OF SCIENCES

ISSN: 2321-5496

Vol 5. Issue 5 . 2017

Research Article

BIOPROSPECTING OF *MUNTINGIA CALABURA*: BIOACTIVE COMPOUNDS AND ITS ANTIOXIDANT, ANTIMICROBIAL AND ANTHELMENTHIC ACTIVITY.

MAHADEVAPPA PARAMESHA[1,2*], TRIVENI TG[2], RAMESH CK[3], NATARAJ S[3]

[1]Plant cell Biotechnology Department, CFTRI, Mysore-570020, Karnataka, India, [2] Department of Biotechnology and Bioinformatics, G M Institute of Technology, Visvesvaraya Technological University, Davangere-577006, Karnataka, India.[3]Department of Biotechnology, Sahyadri Science College, Shivamogga - 577203, Karnataka, India.

Email : paramesham@cftri.res.in

Received:9 November 2016, Revised and Accepted:17 August 2017

ABSTRACT

Objective: *Muntingia calabura* is widely cultivated and has become one of the common roadside trees in most parts of the world. The present study aimed to evaluate medicinal property of leaves and fruits of *M. calabura,* by using antioxidant, antimicrobial and antihelmenthic activities for methanol crude extract.

Methods: Standard methods were used to evaluate secondary metabolites in methanol crude extract of leaves and fruits of *M. calabura.* Total phenolic contents (TPC) were evaluated according to Folin-Ciocalteu method. The free radical quenching ability of extracts were explored by various *in vitro* assays, such as DPPH, hydroxyl radical scavenging and reducing power assay. Additionally, the antimicrobial and anthelminthic activity was conducted to evaluate the biological efficiency of the plant extract**.**

Results: Qualitative phytochemical analysis revealed the presence of alkaloids, saponins, tannins, glycosides, flavonoids and phenols. The reduction of free radical content were observed in dose dependent manner in all tested methods for both leaves and fruits methanol extract of the plant. Further, the antimicrobial activity of plant extract indicates the region where tested microorganisms failed to thrive and the methanol extract also showed evidence to have anthelminthic property.

Conclusion: The presence of secondary metabolites and biological activity of methanol crude extract of leaves and fruits of *M. calabura* ensure the pharmaceutical importance.

Keywords: *Muntingia calabura;* Antioxidant; Antimicrobial activity; Anthelimentic activity.

INTRODUCTION

Muntingia calabura is native to the American continent and is widely cultivated in warm areas of Asian region [1], gardens and along roadsides for ornamental and edible purposes in southern Taiwan [2]. Various parts of this tree have documented for its medicinal uses in both Southeast Asia and tropical America [3, 4]. The roots have been employed as an emmenogogue and the flowers of this species have been used to treat headaches, and as an antidyspeptic, antispasmodic and diaphoretic. Infusions of the flowers of this plant are drunk as a tranquillizer and tonic in Colombia [3]. Phytochemical studies of various parts of this plant have identified many bioactive flavonoids, chalcones, sesquiterpene and phenolic compounds [3-7]. It has been shown that phytochmicals present in various plants exert beneficial effects on cardiovascular diseases such as stroke, coronary artery disease, atherosclerosis and hypertension [8]. These beneficial effects have been partly attributed to their ability to modulate nitric oxide (NO) pathways [9].

Currently the pharmaceutical industries are facing many challenges and favoring the use of plant natural products over the current chemo-clinical drugs available for the treatment of different diseases. Development of resistance to commercial antimicrobial drugs due to abuse of these drugs, the re-emergence of dangerous infectious diseases [10], high production costs and limited effective life span of the synthetic therapeutic agents [11] are important factors that have encouraged a widespread interest in drugs derived from plant extracts. The objective of the present study is to evaluate total phenolics, antioxidants capacity, antimicrobial and anthelminthic activity in *Muntingia calabura*.

MATERIALS AND METHODS

Collection and Extraction of plant material

The leaves and fruits were collected from its natural habitat in vidhyanagar, Shivamogga, Karnataka and authenticated at the Botany Department, Sahyadri Science College, Kuvempu University, Shivamogga, Karnataka. Leaves and fruits were washed, and rinsed with water to remove all the dirt and unwanted particles and then ground into small particles, weighed and mixed with methanol and were incubated for 7 days with occasional shaking. After one week incubation the mixture was filtered using Whatman No. 1 filter paper and the filtrate was concentrated under reduced pressure using a rotary evaporator (Buchi, Rota Vapor, R-205, Germen). The obtained residue was dried and weighed. The dried material left off after evaporation were used for further studies.

Preliminary phytochemical screening

The methanol extract of *M. calabura* was screened for the presence of various phyto-constituents like steroids, alkaloids, glycosides, flavonoids, carbohydrates, amino acids, proteins and phenolic compounds as described by Kokate et al. (1990) [12].

Antioxidant activity

Determination of total phenol content

The total phenol content of extracts was determined using the Folin-Ciocalteu method [13]. The extracts were oxidized with Folin-

Ciocalteu reagent and were neutralized with sodium carbonate. The absorbance of the resulting blue colour measured at 765 nm after 20 min, using gallic acid as standard. Total phenol content was expressed as µg gallic acid equivalent/mg of extract.

DPPH radical scavenging assay

DPPH free radical scavenging activity of the extracts were determined by Sievers, *et al*. (2000) method [14]. Five different concentrations ranging from 50- 250 µg/ml of plant extracts were added to 4 ml of a 0.004% methanol solution of DPPH. After 30minutes of incubation in dark at room temperature, the absorbance was recorded at 517nm. A control reading was obtained using methanol instead of the extract.

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity was measured by Fenton reaction [15] (Halliwell et al.1987). The reaction mixture contained 60µl 1.0mM $FeCl_3$, 90µl 1mM 1,10-phenanthroline, 2.4ml 0.2M-phosphate buffer (pH 7.8), 150µl 0.17 M H_2O_2 and 1ml of extract at various concentrations (50- 250 µg/ml). After incubation at room temperature for 5 minutes, the absorbance was recorded at 560nm.

Reducing power assay

The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu, (1986) [16] with slight modification**.** The reaction mixture contains, 1 ml of extract solution (50- 250 µg/ml) with equal volume of phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide and placed in water bath at 50ºC for 20 min. Then it was cooled rapidly and 1 ml of 10% trichloroacetic acid was added and vortexed centrifuged at 800g for 10 min and its 1.5 ml supernatant was mixed with equal volume of distilled water and 1 ml of 0.1% ferric chloride and left for 10 minutes incubation and absorbance was read at 700 nm.

Ascorbic acid was used as the standard control for all above mentioned antioxidant properties and the percentage inhibition activity was calculated using the following formula from the optical density of the treated and control samples. For the reducing property of test sample was standardized against ascorbic acid.

Percentage (%) Inhibition = $((A_0-A_1)/A_0 \times 100)$

Where, A_0 = absorbance of the control (without test samples) and A_1 = absorbance of test samples.

Antimicrobial screening

The antimicrobial property was carried out by agar well diffusion method [17] (Paramesha et al. 2009). The bacterial strain *Escherichia Coli, Bacillus subtillus, Pseudomonas aerogenosa, Bacillus cereus* and fungal strains *Trichoderma viride, Cladospora Spp, Candida albicans, and Aspergillus niger* were collected from Department of Biotechnology, Sahyadri Science College, Shivamogga, Karnataka, India. The various concentrations of extracts were obtained by dissolving in DMSO. Pure cultures of the organisms were inoculated onto nutrient/potato dextrose broth and incubated for 24 hrs and 48hrs for bacteria and fungal pathogen at 37° C respectively.

The antibacterial assessment initiated by making the lawn of each test bacteria on nutrient agar plates with the help of sterile cotton swab. Well of 0.5 cm in diameter was punched on the plate with the help of sterile cork borer and filled with four different concentrations of extract (2-8%). Ciprofloxacin (2%) was used as reference standard and solvent as control. Plates were incubated at 37°C for 24hrs. The experiment was carried out in triplicate and the zone of inhibition were expressed in millimeter.

The antifungal property was carried out by potato dextrose agar well diffusion method**.** The 0.5mm well was filled with four different concentration of plant extract (5-20%). Fluconazole (5%) was used as reference standard and solvent as control. Plates were observed after 48hrs incubation at 37°C. The experiment was carried out in triplicate and the zone of inhibition were expressed in millimeter.

Anthelmintic activity

The anthelmintic activity was conducted on adult Indian earthworm, *Pheritima posthuma* due to its anatomical and physiological resemblance with the intestinal round worms, parasites of human beings [18-19]. Earthworms were collected from Earthworm Raring Center, Dummalli, Shivamogga (D), Karnataka State and washed with normal saline to remove all fecal matter. Five serial suspensions of each extracts (fruit and leaf extracts) were prepared in DMSO ranging from 0.2% to 1.0 % (2mg/ml to 10mg/ml). The standard reference drug Albendazole was prepared at 0.2% (2mg/ml). All these groups are maintained in 6% dextrose solution. Thus a total of seven groups comprising five tests for each extract and one negative control (Solvent) and one positive control (Albendazole) were subjected to evaluation of anthelmintic property. Each group consists of six approximately equal sized earthworms (20 to 25cm), which were released in to a large petriplate containing 50ml of each suspension. The method of Paramesha *et al.* (2009) [17] was followed for anthelmintic screening. Observations were made for the time taken to paralyze, and death of individual worms. Paralysis was said to occur when no movement of any sort could be observed except the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colors. The experiment was carried out in triplicate.

RESULTS AND DISCUSSION

The phytochemical constituents present in *M. calabura* plant extracts are shown in Table 1. The phytochemical study revealed the presence of alkaloids, tannins, glycosides, flavonoids etc.

Table 1 : Results of phytoconstituents analysis of *Muntingia calabura*

Tests	leaves extract	fruit extract
Alkaloids	++++	+++
Flavonoids	+++	++
Terpenoids	+	+
Saponins	+	
Tannins	+++	+++
Phenolics	+++	++
Cardiac Glycosides	+	++

+ve Presence and –ve indicates the absence and Number of +ve or –e indicates the intensity of phytoconstituent in the extract

Determination of total phenolic content

Phenolic compounds are the principal antioxidant constituents of natural products and are mainly composed of phenolic acids and tannins, which are potent radical terminators. They donate hydrogen to free radicals and break the reaction of lipid oxidation at the initiation step. The total phenolic content determination in an extracts is considered necessary to understand the potentiality of plants for disease prevention and it is the first step in the determination of the antioxidant competence of herbal extracts [20]. The total phenolic content of the methanolic leaf and fruit extracts were found to be 175µg and 130µg gallic acid equivalent/mg respectively.

DPPH radical scavenging assay

The assay is based on the ability of an antioxidants present in the sample to decolorize the DPPH free radical by virtue of their scavenging activities. The DPPH radical contains an odd electron that is responsible for the absorbance at 517 nm and also for the visible deep purple colour [21]. In the present study, the comparison of the antioxidant property of the extract and the reference standard ascorbic acid were studied and results were showed in Fig.1a**.** The result revealed the more effectiveness of the methanolic leaf extract (Ic50 at 46µg/mL) than fruit extract (Ic50 at 123µg/mL) and result of leaves extract was comparable with the ascorbic acid (Ic50 35.5µg/mL). The both extracts were exhibited a significant dose-dependent inhibition of DPPH activity.

Hydroxyl radical scavenging assay

Hydroxyl radical (OH^-) is the most reactive free radical known and can react with everything in living organisms [22]**.** These short-lived species can hydroxylate DNA, proteins, and lipids. It can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions such as copper or iron. It is possible to prevent the

formation of hydroxyl radicals by either deactivating free metal ions [e.g., Fe(II)] through chelation or converting H_2O_2 to other harmless compounds (such as water and oxygen). In the present investigation, the potential scavenging abilities of methanolic extracts might be due to the active hydrogen donor ability of hydroxyl substitution or may be the metal chelating ability with high molecular weight and the proximity of many aromatic rings and hydroxyl groups by specific functional groups or its ability to compete with phenanthroline for radicals [22-23]**.** The activity of methanolic extract in the reaction mixture could able to remove hydroxyl radicals and prevented the degradation of 2-deoxy-2-ribose. The results of Hydroxyl radical scavenging assay is depicted in Fig 1b.

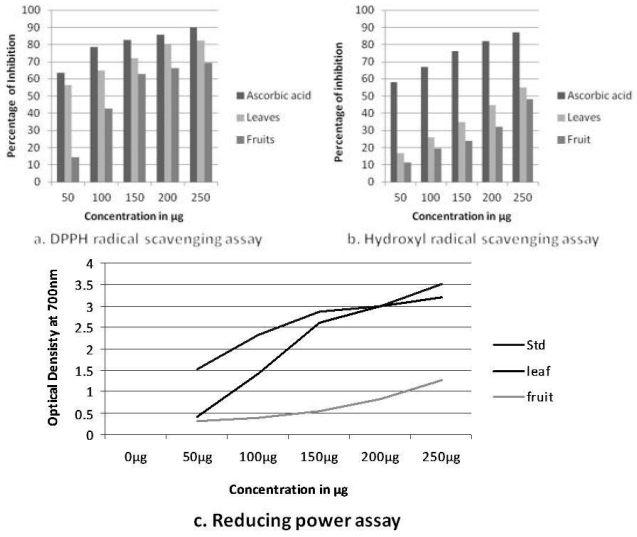


Fig. 1: (a-c). The graphical representation antioxidant assays.

Reducing power assay

The reducing ability of a compound generally depends on the presence of reductants [24], which have been exhibited antioxidative potential by breaking the free radical chain (Fe^{3+} was transformed to Fe^{2+}), by donating a hydrogen atom [25]. The reductive capabilities of the methanolic leaves and fruit extract compared with standard ascorbic acid. Both the tested samples showed very potent reducing capacity. The reducing power of the leaves and fruit extract of *M. calabura* increased gradually with a rise in the concentration **(**Fig 1c**).** Even though, the tested samples recorded slightly lesser reductive activity than the standard, ascorbic acid it is evident from the figure that they could able to reduce the Fe^{3+} ions considerably indicating their reducing capacity.

Antimicrobial screening

The results of antimicrobial activity were showed by zone of inhibition, which revealed that the antimicrobial capacity of methanolic leaves and fruits extract at a concentration dependent manner against the tested organisms at concentrations of 2 - 8% for antibacterial activity and 5- 20% for antifungal activity (Table 2a and 2b).

In antibacterial activity the results showed that, *B.cereus* (16.5±0.6) was more susceptible for standard drug Ciprofloxacin. *P.auerogenosa* (11.08±0.57) and *E.coli* (9.5±0.42) were more liable for leaves and fruit extract at 8% respectively. Among leaves and fruit extract, leaves extract proved to be more effective than fruit extract (Table 2a). Similarly, in antifungal activity, *Cladospora* sps (15.25±0.55) was more sensitive to leaves extract, meanwhile *T.viridae* (11.05±0.77) showed more susceptible to fruit extract (Tabel 2b). *A.niger* (18.25±1.47) was most sensitive to standard drug fluconozol. From the above data it is apparent that the leaves extract *was* more effective at different concentrations on both bacteria and fungi pathogens (Table 2a & 2b; Fig 2).

Table 2a: **Antibacterial Activity of methanolic leaf and fruit extract of *Muntingia calabura***

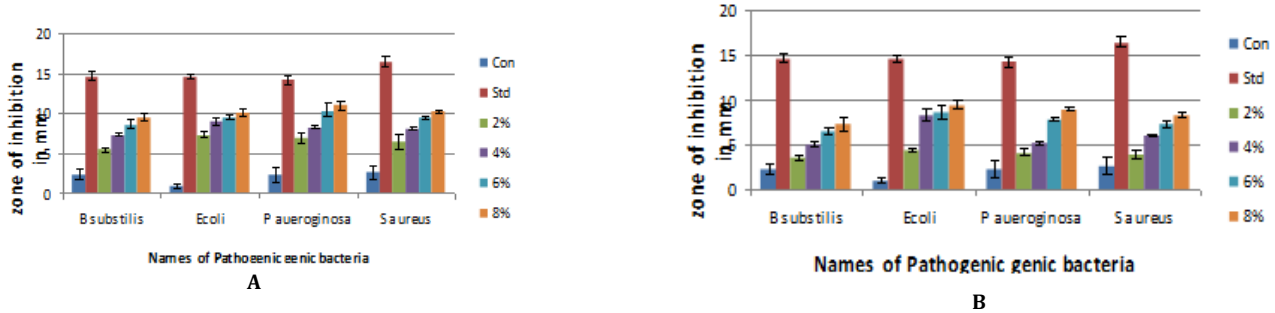
	B.subtilis		***E.coli***		***P.auerogenosa***		***B.cereus***	
	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit
Control	2.33±0.61		1±0.33		2.33±0.98		2.67±0.89	
Ciprofloxin	14.67±0.49		14.67±0.29		14.25±0.55		16.5±0.6	
2%	5.5±0.19	3.58±0.26	7.33±0.39	4.375±0.18	7±0.62	4.1±0.44	6.5±0.95	3.91±0.42
4%	7.33±0.25	5.09±0.29	9.08±0.45	8.25±0.63	8.33±0.21	5.25±0.18	8.16±0.16	6.08±0.13
6%	8.58±0.85	6.56±0.32	9.58±0.33	8.625±0.78	10.26±0.98	7.83±0.23	9.5±0.23	7.25±0.32
8%	9.5±0.49	7.33±0.82	10.16±0.43	9.5±0.42	11.08±0.57	9±0.25	10.25±0.29	8.33±0.29

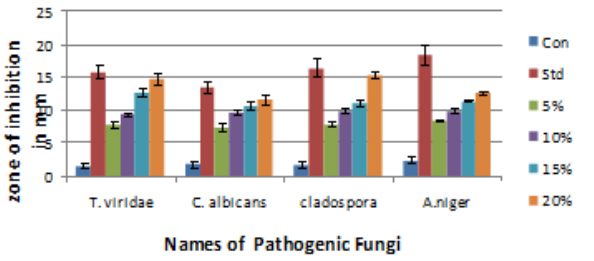
The inhibition zone mentioned in "millimetre" and all the tests are carried out in triplicates.

Table 2b: Antifungal Activity of methanolic leaf and fruit extract of *Muntingia calabura*

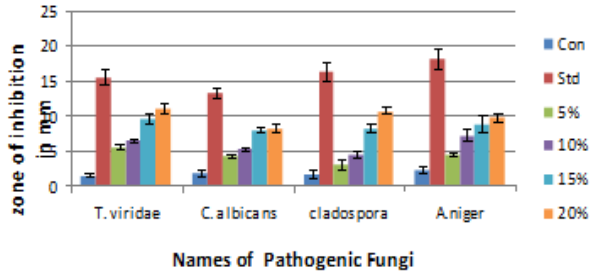
	T. viridae		***C. albicans***		***Cladospora***		***A.niger***	
	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit
Control	1.48±0.31		1.75±0.44		1.65±0.58		2.3±0.46	
Fluconozol	15.64±1.1		13.33±0.91		16.33±1.33		18.25±1.47	
2%	7.75±0.39	5.47±0.39	7.25±0.62	4.21±0.32	7.75±0.33	3±0.66	8.25±0.22	4.5±0.22
4%	9.34±0.3	6.47±0.3	9.5±0.4	5.25±0.25	9.75±0.4	4.5±0.41	9.75±0.44	7.25±0.88
6%	12.67±0.58	9.58±0.58	10.5±0.58	8±0.47	11±0.61	8.25±0.47	11.25±0.08	8.75±1.2
8%	14.6±0.77	11.05±0.77	11.5±0.66	8.25±0.71	15.25±0.55	10.75±0.52	12.55±0.29	9.75±0.44

The inhibition zone mentioned in "millimetre" and all the tests are carried out in triplicat





C



D

Fig 2: Antimicrobial activity of methanolic leaf and fruit extract of Muntingia calabura

Fig 2a. Antibacterial activity of methanolic leaves extract on different pathogenic bacteria.
Fig 2b. Antibacterial activity of methanolic fruit extract on different pathogenic bacteria.
Fig 2c. Antifungal activity of methanolic leaves extract on different pathogenic fungi.
Fig 2d. Antifungal activity of methanolic fruit extract on different pathogenic fungi.
he antimicrobial activity maybe due to the presence of active principles in the plant methanol extract [26]. It acts potent antimicrobial because it contains flavonoids, triterpenoids, alkaloids, steroids, phenolic compounds, tannins, and cardiac glycosides, which have multiple biological effects, including antioxidant, wound healing *etc.* and are toxic to the microorganisms. Flavonoids,phenolic compounds in particular of plant are important for the plant growth and defense against infection and injury. These compounds while exhibiting antioxidant property are usually also act as good antimicrobial agents [17, 27-29]. The underlying mechanisms could be enzyme inhibition by oxidation [11]. Further, the variation in antimicrobial sensitivity among bacteria/fungi may be due to the differences in the chemical nature of the cell wall and cell membrane of each micro organism [17].

Anthelmintic activity

Most anthelmintics target the neuro-musculature and therefore similarities here become of particular importance. The wiring diagram for the neuromuscular system is similar between Pheritima *posthuma* and the parasitic nematode *Ascaris suum* [17, 30] and in the major neurotransmitters; acetylcholine in the excitatory motorneurones, GABA in the inhibitory motor neurons [31] and glutamate providing input onto the motor neurons [32].
The result of the anthelmintic activity of leaves and fruits methanolic extract of *M. calabura* for two parameters *viz,* time of paralysis and time of death are presented in Table 3. The effective and maximum reduction of time for paralysis was found to be at 10% for both. The leaves and fruits extract possessed potent anthelmintic activity at a concentration of 2-10%. Comparison of mean values of five different concentrations v/s standard (10.52 min) revealed that the concentration above 6% up to 10% , were effective in terms of time required for paralysis and maximum reduction was found to be at 10% (7.59 and 13.18 min for leaf and fruit extract respectively) (Table 3). And the concentrations above 6% of the test have exhibited shorter duration for the time of death than the standard (14.52 min) wherein highest reduction for the time of death has been recorded at 10% (10.23 and 42.02 min for leaf and fruit extract respectively), indicating the potency of the extracts for anthelmintic activity.

Tabel 3: Anthelmintic activity of Methanolic leaf and fruit extract of *Muntingia calabura*

Sl. No	Dosage.	Time of paralysis		Time of death	
		Leaves	Fruit	Leaves	Fruit
1	Std	10.52		14.52	
2	2%	12.4	37.1	14.3	55.56
3	4%	11.22	30	13.43	53.53
4	6%	10.52	21.5	13.07	46.9
5	8%	9.42	15.4	11.58	44.55
6	10%	7.59	13.18	10.23	42.02

All the tests are carried out in triplicates.

CONCLUSION

In the present investigation, the leaves and fruit methanolic extract of *M. calabura* be evidence for its good antioxidant activity and also to be good antimicrobial and anthelmenthic activity. The synergistic effect of methanolic extract of *M. calabura* leaves demonstrates the potential of this plant as a candidate for antibiotic-resistance–modifying compounds. Hence, this plant warrants further study in the manner to fractionate the extract, isolation and identification of the compounds responsible for the present result. An elucidation of the mechanism of action of the compounds to determine the therapeutic applicability of such compounds in combination therapy is a subject in need of further study.

REFERENCE

1. Chin WY. A guide to the wayside trees of Singapore. BP Singapore Science Centre; 1989. p. 145.
2. Liu TS, Lo HC. Tiliaceae in Flora of Taiwan. 2nd edn. Editorial Committee of the Flora of Taiwan, Taipei, Taiwan: 1993. Vol 3: p 730-2.
3. Kaneda N, Pezzuto JM, Soejarto DD, Kinghorn AD, Farnwort NR, Santisuk T, *et al.* Plant anticancer agents, XLVII. New cytotoxic flavanoids from *Muntingia calabura* roots. J. Nat. Prod 1991 (54): 196-206.
4. Nshimo CM, Pezzuto JM, Kinghorn AD, Farnsworth NR. Cytotoxic constituents of *Muntingia calabura* leaves and stems collected in Thailand. Int. J. Pharmacol 1993 (31): 77-81.
5. Wong KC, Chee SG, Er CC. Volatile Constituents of the Fruits of *Muntingia calabura* L. J Essent Oil Res 1996 (8): 423–426.
6. Su BN, Park EJ, Vigo JS, Graham JG, Cabieses F, Fong HH, Pezzuto JM, Kinghorn AD. Activity-guided isolation of the chemical constituents of *Muntingia*

calabura using a quinone reductase induction assay. Phytochemistry 2003. 63(3):335-41.

7. Chen JJ, Lee HH, Duh CY, Chen IS. Cytotoxic chalcones and flavonoids from the leaves of *Muntingia calabura*. Planta Med 2005; 71(10):970-3.
8. Wang HX, Ng TB. Natural products with hypoglycemic, hypotensive, hypocholesterolemic, antiatherosclerotic and antithrombotic activities. Life sci 1999;65(25):2663-77.
9. Achike FI, Kwan CY. Nitric oxide, human diseases and the herbal products that affect the nitric oxide signalling pathway. Clin Exp Pharmacol Physiol. 2003; 30(9):605-15.
10. Rojas R, Bustamante B, Bauer J, Fernández I, Albán J, Lock O. Antimicrobial activity of selected Peruvian medicinal plants. J Ethnopharmacol 2003;88(2):199-204.
11. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999; 12(4):564-82.
12. Kokate CK, Purohith AP, Gokhale SB. Pharmacognosy, Pune, India, Nirali Prakashan; 1990.
13. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J Agric Food Chem* 1995; 43(1):27-32.
14. Sievers A, Oshinowo L, Schultze W, Koch A, Richter R. Einfache dünnschicht-chromatographische Prüfung auf antioxidative Verbindungen mit dem DPPH-Test. C35. Camag Bibliography Service. 2000;88:14-5.
15. Halliwell B, Gutteridge JM, Aruoma OI. The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. Anal biochem 1987;165(1):215-9.
16. Oyaizu M. Studies on products of browning reaction--antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr 1986.
17. Paramesha M, Ramesh C, Krishna V. Studies on anthelmintic, antibacterial and antiviral activity of methanol extract from leaves of *Carthamus tinctorius* L. Bioscan. 2009;4:301-4.
18. Vidyarthi RD. A Textbook of Zoology, 14th ed., New Delhi, India, S. Chand and Co. 1977.
19. Vigar Z. Atlas of Medical Parasitology. 2nd Edn. P.G. Publishing House, Singapore. 1984. p. 216-217.
20. Tosun M, Ercisli S, Sengul M, Ozer H, Polat T, Ozturk E. Antioxidant properties and total phenolic content of eight Salvia species from Turkey. Biol Res 2009; 42(2):175-81.
21. Paramesha M, Ramesh CK, Krishna V, Kumar YS, Parvathi KM. Hepatoprotective and in vitro antioxidant effect of Carthamus tinctorious L, var Annigeri-2-, an oil-yielding crop, against CCl 4-induced liver injury in rats. Pharmacogn Mag 2011 (28):289.
22. Li H, Wang X, Li P, Li Y, Wang H. Comparative study of antioxidant activity of grape (*Vitis vinifera*) seed powder assessed by different methods. J Food Drug Anal 2008;16(6):67–73.
23. Aurand LW, Boone NH, Giddings GG. Superoxide and singlet oxygen in milk lipid peroxidation. J Dairy Sci 1977;60(3):363-9.
24. Duh PD, Tu YY, Yen GC (). Antioxidant activity of aqueous extract of *harn jyur* (*Chyrsanthemum morifolium* Ramat). Lebensm-wiss Technol 1999. 32: 269-277.
25. Gordon MH. The mechanism of antioxidant action in vitro. In Food antioxidants. Springer Netherlands 1990. p. 1-18.
26. Ghosh T, Maity TK, Swain PK, Bose A. Anthelmintic and antimicrobial activity of *Enhydra fluctuanslour*. aerial parts. Pharmacogn Mag 2007;3(11):204–208.
27. Cos P, Calomme M, Sindambiwe JB, De Bruyne T, Cimanga K, Pieters L, et al. Cytotoxicity and lipid peroxidation-inhibiting activity of flavonoids. Planta med 2001;67 (06):515-9.
28. McGaw LJ, Jäger AK, Van Staden J, Eloff JN. Variation in antibacterial activity of Schotia species. S Afr J Bot 2002;68(1):41-6.
29. Kotze M, Eloff JN, Houghton PJ. Extraction of antibacterial compounds from *Combretum microphyllum* (Combretaceae). S Afr J Bot 2002;68 (1):62-7.
30. Angstadt JD, Donmoyer JE, Stretton AO. Retrovesicular ganglion of the nematode *Ascaris*. J Comp Neurol 1989;284(3):374-88.
31. Johnson CD, Stretton AO. GABA-immunoreactivity in inhibitory motor neurons of the nematode Ascaris. The Journal of neuroscience 1987;7(1):223-35.
32. Davis RE. Neurophysiology of glutamatergic signalling and anthelmintic action in *Ascaris suum*: pharmacological evidence for a kainate receptor. Parasitology. 1998;116 (5):471-86.