The Pharma Innovation Journal 2017; 6(12): 223-228

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating 2017: 5.03
TPI 2017; 6(12): 223-228

www.thepharmajournal.com
Received: 10-10-2017
Accepted: 11-11-2017

Aijaz Ahmad Ganaie
Environmental Biotechnology Lab, Deptt. of P.G Studies and Research in Biological sciences, R. D. University, Jabalpur, Madhya Pradesh, India

Correspondence
Aijaz Ahmad Ganaie
Environmental Biotechnology Lab, Deptt. of P.G Studies and Research in Biological sciences, R. D. University, Jabalpur, Madhya Pradesh, India

Antioxidant and Antimicrobial activity of a few concentrates of *Puccinella kashmirians:* A rare plant of Kashmir

Aijaz Ahmad Ganaie

Abstract
Introduction: *Puccinella kashmirians* is an uncommon plant found in Kashmir valley.
Techniques: Antimicrobial movement of the of the plant exracts were done against different bacterial and parasitic strains like: *E. coli* (MTCC 407), *P. aeruginosa* (MTCC 139*), S. aureus* (MTCC 96), *B. subtilis* (MTCC 441), *K. pneumonia* (MTCC 49) ; *A. niger* (MTCC1344), *P. crysogenum* (MTCC 947), *C. albicans* (MTCC), *T. rubrum* (MTCC 8469), *E. floccosum* (MTCC 613), *M. canis* (MTCC 296). Different oxidation prevention agent strategies were utilized for assessment of free radical rummaging movement of the plant extricates, IC50 esteems were observed to be bringing down with expanded extremity.
Results: The plant under examination was found to have wide range antimicrobial movement against different bacterial and parasitic strains The plant additionally demonstrates a huge radical searching action with IC_{50} estimations of 98.94 124.63, 98.63, 191.88, 100.79 µg/ml in methanolic remove.
Conclusion: The plant can likewise fill in as potential contrasting option to treat different infections, as the plant was found to have wide range antimicrobial potential and high antioxidant potential.

Keywords: *Puccinella kashmirians; E. floccosum*

1. Introduction
Man has been ever covetous of learning to investigate numerous new things yet numerous things stay hid for some eras to investigate them, today even after drawn out stretch of time with so much logical and innovative headway, the earths widely varied vegetation are as yet remaining the most critical wellspring of potential medications for customary hakims. Kashmir is a vertical supply of plants bearing restorative esteem. Kashmir one of only a handful couple of conditions of our nation where sweet-smelling plants of numerous types can be developed due to the reasonable climatic conditions differ from mild fields to tropical areas, hills, valleys, irrigated soil and so forth. In Kashmir the utilization of a few plants pieces to cure particular pains has been in endeavor from antiquated times. This arrangement of prescription gives the necessities of around half individuals of our populace especially those living in towns along the sloping ranges of our valley for its restorative incentive as well as give methods for vocation to many individuals engaged with their cultivation. Use of plants for curing of maladies is not limited to specialist just but rather is referred to numerous family units too and our insight into therapeutic plants has for the most part been acquired tradionally. There is a developing need on everywhere throughout the world to move to normal based items including restorative plants (Non-lethal) from engineered (Toxic) Some of the regular therapeutically critical parts confined from plants resemble terpinols, steroids, cortisones, Colchine, limonine, flavoinoids, podophylltoxin and so on. Nair, (1994). The parts of a plant that guide in curing different sicknesses are reffered to as 'dynamic principals'. The main gatherings of dynamic principals incorporate steroids, terpenoids, fundamental oils, glycosides, tannins and soon.
Free radicals era is an ordinary marvel in natural framework. Here and there due to over era, body's guard instrument is not ready to evacuate them, and subsequently a condition called oxidative anxiety is produced in the body. The free radicals are the substance species, which have an unpaired electron and are consequently differ shaky and responsive. Keeping in mind the end goal to achieve steadiness they respond with their neighboring iotas to pick up the electrons coming about the era of new free radicals, which thusly assault to other adjacent atoms causing a web of responses. Vigorous life form utilization of oxygen to oxidize nourishment and get the energy, a wonder basic for their sustenance.

In any case, amid this oxidation procedure the oxygen particle itself get diminished and from a middle of the road called as the receptive oxygen species (ROS). The part of free radical response in the science has turned into a territory of serious intrigue. It is for the most part acknowledged that free radicals assume a vital part in the advancement of tissue harm and obsessive occasions in living life forms. There is expanding enthusiasm for the regular cancer prevention agents contained in the restorative and the dietary plants, which are the possibility for the counteractive action of oxidative anxiety or harm.

2. Material and Methods

Puccinella kashmirians was gathered from its regular source in Kashmir. After distinguishing proof of the plant from dept. of botany Islamia college, Srinagar, the leaves of the plant were brought and altogether washed with tap water, flushed a couple of times in refined water and after that dried in shade. The dried leaves were taken and removed into little pieces, powdered in a processor and after that separated with petroleum- ether, ethyl acetate derivation and methanol.

2.1 Preparation of Plant Extracts

An institutionalized dissolvable extraction convention was utilized. 50 g of plant powder was encouraged to a Soxhlet extractor fitted with a 0.5 L round-base cup and a condenser. Extraction was done serially with various solvents utilizing pet. ether, ethyl acetate derivation and methanol. The extraction was executed on a water shower for 12 hrs with 0.4 L of every dissolvable. After fulfillment of extraction, the dissolvable was refined off in a rotating evaporator at 35-45 ^{o}C. The dried concentrates were weighed to decide the yield of dissolvable constituents. Every one of the concentrates of the plant were put away under cooler (4 ^{o}C), until utilized for additionally examinations.

2.2 Determination of Total phenolic content

The aggregate substance of solvent phenolic mixes in plant separates was resolved with Folin Ciocalteau reagent (FCR) as indicated by the technique portrayed in (Chang *et al*. 2002; Roy *et al*, 2010) [4, 15]. Gallic corrosive was utilized as a standard. 0.5 ml of each concentrate with a centralization of 5 mg/ml was independently blended with Folin–Ciocalteu reagent (0.2 N, 2.5 ml) and watery Na_2CO_3 (1 M, 2 ml) arrangement. The response blend was permitted to remain at room temperature for 15 min. The absorbance was measured at 765 nm utilizing an UV–visible spectrophotometer. The adjustment bend (Slope = 57.44 ± 1.10; R2 = 0.997) was readied utilizing arrangements of gallic corrosive (standard) in methanol: water blend (50:50, v/v) with focuses running from 0–30 µg ml^{-1}. The aggregate polyphenol content was communicated as far as milligram of gallic acid proportionate per gram of dry mass (mg GAE g^{-1}). Three recreates were performed for each specimen concentra focus to check the reproducibility of the test result and to get more exact outcomes.

2.3 Determination of Total Flavonoid Content

The Aluminum chloride colorimetric strategy was utilized for flavonoid content assurance of each concentrate (Roy *et al*, 2010; Mcdonald *et al* 2001) [15, 10]. 0.5 ml of each concentrate (5 mg ml-1) was independently blended with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetic acid derivation and 2.8 ml of refined water. The response blend was kept at room temperature for 30 min. The absorbance of the response blend was measured at 415 nm with an UV–visible spectrophotometer. The adjustment bend (Slope = 65.68 ± 0.76; R2 = 0.999) was recorded by utilizing quercetin (standard) arrangements in methanol with fixations running from 0 to 20 µgml-^{1}. The aggregate flavonoid content was communicated as far as milligram of quercetin identical per gram of dry mass (mg QE g^{-1}).

2.4 Phytochemical screening

The phytochemical screening tests of the different plant extracts were performed by using standard procedures (Sofowora 1993; Ayoola *et al*. 2008)

2.5 Evaluation of Antioxidant Activity

The antioxidant activities were performed using assays viz. DPPH radical scavenging assay, Ferric ion reducing assay, FRAP assay, Hydrogen peroxide scavenging assay and Lipid per-oxidation assay. The methods of these assays are described below:

Modified DPPH (Shameem *et al.,* 2015), Ferric (Fe^{3+}) reducing power assay (Yildirim *et al*., 2000), FRAP assay (Ruch *et al*. 1989) [16], hydrogen peroxide scavenging assay (Feerreira *et al*, 2010) and Lipid per-oxidation Method (Padmaja *et al*., 2011) [11] were followed for determining antioxidant activity.

2.6 Antimicrobial Activity

The cleansed lectin was screened for their antimicrobial action by utilizing agar well method (Barry., 1980) [2] by measuring the measurement of the inhibitory zones in mm utilizing diverse grouping of decontaminated lectin in methanol. The distances across of the zones of restraints of the examples were than contrasted and the breadth of the zone of hindrance delivered by the standard anti-toxin, for example, ciprofloxacin (antibacterial) and fluconozol (antifingal). Supplements agar medium and potato dextrose agar were utilized for deciding antibacterial and antifungal exercises individually.

3. Results and Discussion

The yields of various extracts of the plants under study are given in Table 1. All the extracts were obtained as dark-green semi-solid material.

Table 1: Yields of various extracts of plant material (in grams).

Name of Plant	Pet ether Extract	Ethyl acetate Extract	Methanol Extract
Puccinella kashmirians	1.346	2.051	3.106

3.1 Phytochemical screening

Diverse strategies were taken after to decide subjectively the nearness of phytochemical constituents show in the plant methanol remove. The measure of unrefined concentrates shifted among the solvents utilized. Under the present investigation, the methanol separate (3.106 g) demonstrated higher yield. The subjective phytochemical screening of rough concentrates of *Puccinella kashmirians* uncovered that alkaloids, phenols, anthraquinones, and flavonoids were available. Saponins, glycosides, and +tannins were missing in both the RP and AP separates while terpenoids were available in just the RP remove (Table 2).

Table 2: Phytochemical analysis of different secondary metabolites present in the aerial extracts of *Puccinella kashmirians.*

Phytochemical constituents	Pt. ether	Ethyl acetate	Methanol. Extract
Alkaloids	+	+	+
Glycosides	+	+	+
Anthraquinones	+	+	+
Saponins	−	+	+
Tannins	−	−	-
Terpenoids	−	+	+
Flavonoids	+	+	+
Phenolic compounds	+	+	+
Yield (%)	1.3	2.05	3.1

(+) = present, (−) = absent

The outcomes got in the present investigation uncovered that the level of the aggregate phenolic (TP) substance and aggregate flavonoid content in various concentrates of the chose plant was impressive. The TP content was measured by Folin-Ciocalteu reagent as far as gallic corrosive proportional (standard bend condition: (Slope = 57.44 ± 1.10; R2 = 0.997)). The TP substance of various concentrates regarding GAE µg/ml is appeared in Table 3. Among the concentrates methanol remove demonstrates most astounding phenolic content.

The TF content was measured as far as quercetin proportional (The alignment bend (Slope = 65.68 ± 0.76; R2 = 0.999)). The TF substance of various concentrates as far as QE µg/ml is appeared in Table 4. Among the concentrates methanol extricate indicates most astounding flavinoidal content.

Table 3: Total Phenolic content of the plant under study, expressed as gallic acid equivalents in µg/ml for 100 g of extract.

Plant name	Total Phenolic Content		
	Gallic Acid Equivalent) (µg/ml for 100 g of extract)		
	Pet. ether Extract	Ethylacetate Extract	Methanol Extract
Puccinella kashmirians	23.83	665.08	844.56

Table 4: Total flavonoid content of the plants under study, expressed as Quercetin equivalents in µg/ml for 100 g of extract.

Plant name	Total FlavonoidContent		
	Quercetin Equivalent) (µg/ml for 100 g of extract)		
	Pet. ether Extract	Ethylacetate Extract	Methanol Extract
Puccinella kashmirians	8.87	664.24	1004.84

3.2 Antioxidant Activity

3.2.1 DPPH radical scavenging assay

DPPH test is a standout amongst the most generally utilized strategy for screening cell reinforcement potential. DPPH being a steady free radical at room temperature and acknowledges an electron or hydrogen radical to end up plainly stable diamagnetic particle. The lessening capacity of DPPH radical was controlled by diminish in its absorbance at 517 nm, which is initiated by various cell reinforcements. The decline in absorbance of DPPH radical caused by antioxidants as a result of the response between antioxidant and radical advance which brings about the rummaging of the radical by hydrogen gift. It is outwardly observable as an adjustment in shading from purple to yellow. The exercises acquired as a component of different centralizations of various concentrates are displayed (Figure 1). It is clear from the assume that the methanolic remove indicates higher DPPH radical searching movement. The IC_{50} estimations of the concentrates for this action were resolved from the diagram and qualities are given in the Table 5 for all concentrates.

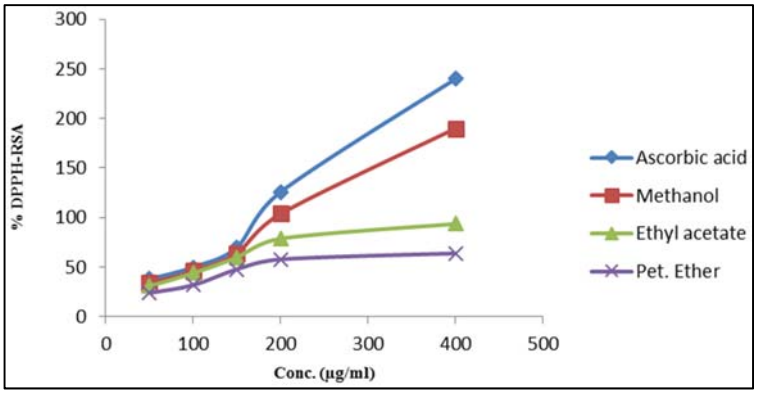

Fig 1: % DPPH Radical Scavenging Activity of various extracts of under study plants and standard (Ascorbic acid).

3.2.2 Ferric (Fe^{3+}) reducing power assay

The reducing ability is also an indirect evidence for the antioxidant activity of an extract or a compound i.e. the decreasing species exhibit in the concentrate causes the diminishment of the Fe^{3+} ferricyanide complex to frame Fe^{2+} particles; this response was checked spectrophotometrically

by recording the absorbance of the response blend at 700 nm (Prasad *et al*., 2010) [12]. The decreasing force qualities of various concentrates is appeared in (Figure 2). From the figure plainly, out of every one of the three concentrates of the plants considered, methanolic separate has most elevated ferric particle diminishing movement at various fixation, trailed by ethyl acetic acid derivation concentrate and slightest for pet. ether extricate. The diminishing force increments with expanding the centralization of concentrates in the arrangements. IC50 esteem is appeared in Table 5.

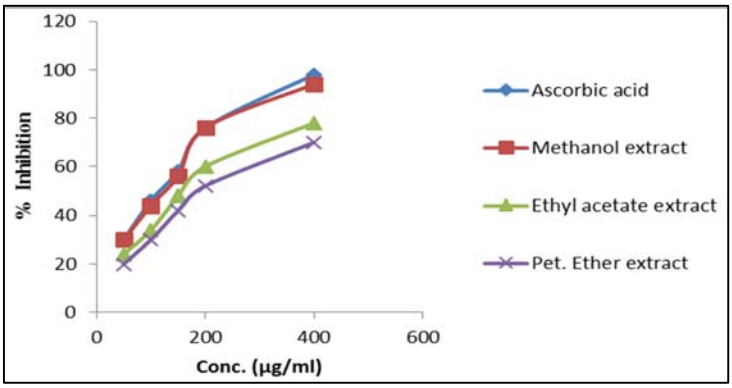

Fig 2: Ferric reducing activity of different extracts of under study plant and standard Ascorbic acid.

FRAP

The ferric reducing antioxidant power (FRAP) assay measures the reducing ability of antioxidants against the oxidative effects of reactive oxygen species. Electron giving cell reinforcements can be portrayed as reductants and inactivation of oxidants by reductants can be depicted as redox responses. This measure depends on the capacity of antioxidant to lessen Fe^{3+} to Fe^{2+} within the sight of tripyridyltriazine (TPTZ), whereby an extreme blue Fe^{2+}–TPTZ complex with an absorbance greatest at 593 nm is framed. Expanding absorbance shows an expansion in reductive capacity. Among the distinctive specimens disengaged from the considered plant at different fixations (50, 100, 150, 200, 400 µg/ml) were analyzed. From Figure 3 obviously there was a focus subordinate increment in diminishing movement in all concentrates and methanol extricates has most astounding action among every one of the concentrates. IC_{50} esteem is displayed in The ferric lessening cell reinforcement control (FRAP) test measures the decreasing capacity of cancer prevention agents against the oxidative impacts of receptive oxygen species. Electron giving cell reinforcements can be portrayed as reductants and inactivation of oxidants by reductants can be depicted as redox responses. This measure depends on the capacity of cell reinforcements to lessen Fe^{3+} to Fe^{2+} within the sight of tripyridyltriazine (TPTZ), whereby an extreme blue Fe^{2+}–TPTZ complex with an absorbance greatest at 593 nm is framed. Expanding absorbance shows an expansion in reductive capacity. Among the distinctive specimens disengaged from the considered plant at different fixations (50, 100, 150, 200, 400 µg/ml) were analyzed. From Figure 3 obviously there was a focus subordinate increment in diminishing movement in all concentrates and methanol extricates has most astounding action among every one of the concentrates. IC_{50} esteem is displayed in Table 5.

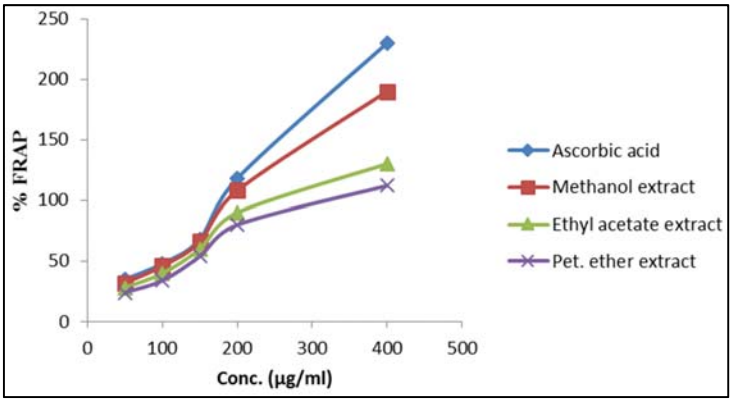

Fig 3: FRAP activity of different extracts of under study plant and standard Ascorbic acid.

Hydrogen peroxide scavenging activity

Hydrogen peroxide is a frail oxidizing specialists and can inactivate a couple of chemicals specifically by the oxidation of fundamental thiol (- SH) gatherings. Hydrogen peroxide can cross cell layer quickly, once inside the cell, H2O2 can most likely respond with each conceivable particle in living being particularly with DNA, Proteins and Lipids and this might be the birthplace of a significant number of lethal impacts. Hydrogen peroxide rummaging action level of different extracs is exhibited in chart (Figure 4) and IC_{50} esteem is introduced in (Table 5). The concentrates demonstrated the focus subordinate rummaging as contrasted and standard ascorbic acid.

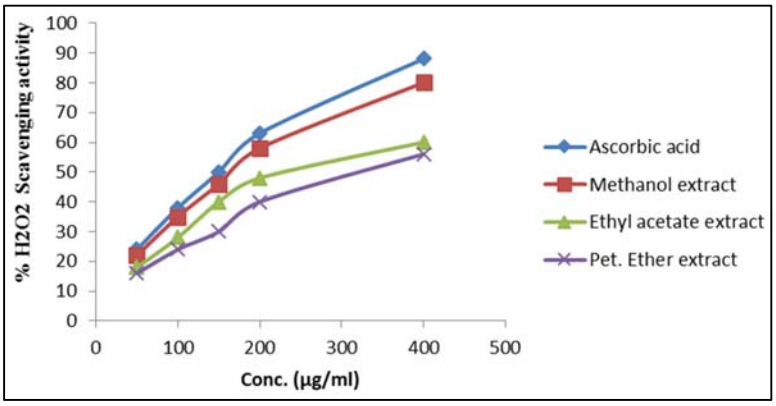

Fig 4: Hydrogen peroxide scavenging activity of various plant extracts and standard Ascorbic acid.

Lipid per-oxidation Method

Lipids Peroxidation has been a noteworthy issue for the rack soundness of nourishments. Because of oxidation of lipids, lipid hydroperoxides framed in the sustenance frameworks are divided or polymerized to shape different optional items. These items are in charge of the mediocrity of the sustenance quality, for example, crumbling of taste and flavor (Spanier *et al.,* 1992; Jensen *et al*., 2001) [17, 8] and diminished nutritious esteem (Ames, 1983) [1]. Lipids Peroxidation action level of different extracs is exhibited in chart (Figure 5) and IC_{50} esteem is introduced in (Table 5). The concentrates demonstrated the focus subordinate action as contrasted and standard ascorbic acid.

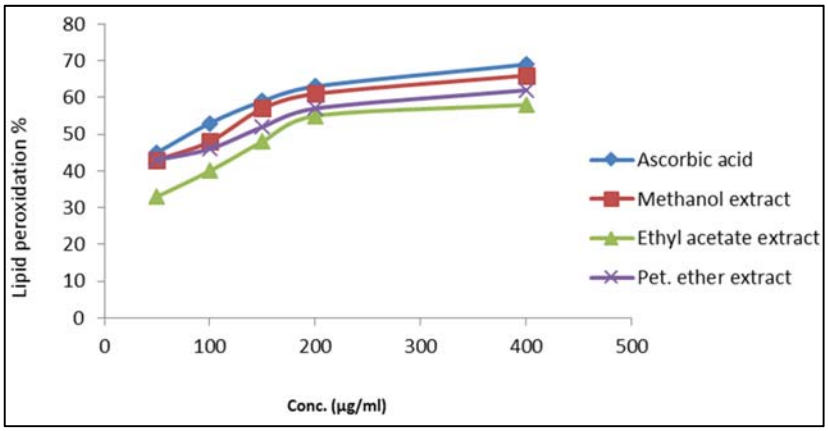

Fig 5: Lipid peroxidation activity of various plant extracts and standard Ascorbic acid.

3.3. Antimicrobial activity

3.3.1 Antibacterial Activity

Examination is being completed of plant material as option wellspring of antimicrobial operator. It has turned out to be more typical in the course of recent years, because of the expanded rate of improvement of anti-infection resistance creature. The restraint of bacterial development in-vitro by the concentrates of plants could be because of the nearness of some dynamic mixes in the concentrates. These dynamic mixes may act alone or in blend to repress bacterial development. It might be because of unrefined plant extricates containing numerous natural parts including flavonoids, tannins, alkaloids, triterpenoids, all of which are known to have antibacterial effects (Santhi *et al*, 2006; Rabe and Staden, 1997) [14].

3.3.2 Antifungal Activity

In our examination all the three concentrates demonstrated noteworthy antifungal property. The methonolic extricate was found to give more steady antibacterial action contrast with different concentrates like oil ether and ethyl acetic acid derivation. The after effects of our examinations are appeared in Table 6.

4. Conclusion

Puccinellia kashmirians is an uncommon therapeutic plant of Kashmir, before this work no such examination has been directed on this plant. The plant indicates amazing antioxidant potential and can be utilized for anticipation or treatment of different oxidative related ailments. The plant can likewise fill in as potential contrasting option to treat different infections, as the plant was found to have expansive range antimicrobial potential.

5. Acknowledgement

The authors express gratitude to the Department of Biological sciences, R.D University, Jabalpur for providing necessary support to carry out this work.

6. References

1. Ames BN. Dietary Carcinogens and Anticarcinogens (Oxygen Radicals and Degenerative Diseases). Science. 1983; 221:1256-1264.
2. Barry AL. Procedures for testing antimicrobial agents in agar media. In: *Antibiotic in laboratory medicine*. (Lorian V ed.). William and Wilkins Co. Baltimore, USA, 1980, 1-23.

3. Bozin B, Mimica N, Simin N, Anakov G. Characterization of the volatile composition of essential oils of some *Lamiaceae* spices and the antimicrobial and antioxidant activities of the entire oils. J. Agri. Food. Chem, 2001; 58:1822-2828.
4. Chang C, Yang M, Wen H. Chern, J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis. 2002; 10:178-182.
5. Christopher J, Morris Earl JR, Trenam CW, Blake DR. Reactive oxygen species and iron-a dangerous partnership in inflammation. The International Journal of Biochemistry & Cell Biology. 1995; 27(2):109-122.
6. Ferreira EB, Fernandes LC, Galende SB, Cortez DAG, Bazotte RB. Hypoglycemic effect of the hydroalcoholic extract of leaves of *Averrhoa carambola* L. (Oxalidaceae). Brazilian Journal of Pharmacognosy. 2008; 18(3):339-343.
7. Harborne JB. Phytochemical methods, London Chapman Hall Ltd, New York, 1973, 49-188.
8. Jensen PN, Sorensen G, Engelsen SB, Bertelsen G. Evaluation of quality changes in walnut kernels (Juglans regia L.) by Vis/NIR spectroscopy. J Agric Food Chem. 2001; 49(12):5790-5796.
9. Matsushige K, Banset P, Kadota S, Namba T. Potent free radical scavenging activity of dicaffeyl quinic acid derivatives from propolis. Traditional Medicines. 1996; 13:217-228.
10. Mcdonald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. *Food Chemistry*. 2001; 73:73-84.
11. Padmaja M, Sravanthi M, Hemalatha KPJ. Evaluation of antioxidant activity of two Indian medicinal plants. Journal of Phytology. 2011; 3(3):86-91.
12. Prasad KN, Xie H, Yang B, Qiu B, Wei X. Antioxidant and anticancer activities of 8-hydroxy psoralen isolated from Wampee [*Clausena lansium* (Lour.) Skeels] peel. Food Chemistry. 2010; 118:62-66.
13. Rabe T, Van- Stadin VJ. Antibacterial activity of South African plants used for medicinal purposes. J. Ethnopharmacol. 1997; 56:81-87.
14. Rabe T, Van Stadin VJ. Antibacterial activity of South African plants used for medicinal purposes. J. Ethnopharmacol. 1997; 56:81-87.
15. Roy N, Mondal S, Laskar RA, Basu S, Mandal D, Begum NA. Biogenic synthesis of Au and Ag nanoparticles by Indian propolis and its constituents. Colloids and Surfaces B: Biointerfaces. 2010; 76:317-325.
16. Ruch RJ, Cheng SJ, Klaunig JF. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989; 10:1003-1008.
17. Spanier AM, Angelo AJ, Shaffer GP. Response of beef flavor to oxygen depletion and an antioxidant/chelator mixture. J Agric Food Chem. 1992; 40(9):1656-1662.