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Mohd Yaseen
a) Department of Chemistry, Saifia Science College Bhopal, Madhya Pradesh, India
b) Department of Clinical Biochemistry, University of Kashmir, Srinagar, Jammu and Kashmir, India

Manzoor Ahmad
Department of Chemistry Saifia Science College Bhopal, Madhya Pradesh, India

Mudasar Ahamd
Phytochemistry Laboratories Centre of Research for Development, Jammu and Kashmir, India

BA Ganai
Department of Clinical, Biochemistry University of Kashmir, Srinagar, Jammu and Kashmir, India

Rashida Qureshi
Department of Chemistry, Saifia Science College, Bhopal, Madhya Pradesh, India

Correspondence
Rashida Qureshi
Department of Chemistry, Saifia Science College, Bhopal, Madhya Pradesh, India

Antioxidant and free radical scavenging properties of *Arictum lappa*

Mohd Yaseen, Manzoor Ahmad, Mudasar Ahamd, BA Ganai and Rashida Qureshi

Abstract
The Methanolic crude extracts of *Arictum Lappa* were screened for their antioxidant free radical scavenging properties using a-tocophorol and butylated hydroxy toluence (BHT) as standard antioxidants. Antioxidant activity was measured by ferric thiocyanate (FTC) assay and compared with the thiobarbiturc acid (TBA) method. Free redical scavenging activity was evbaluatyed using diphenyI picryl hydrazyl (DPPH) radicals. The overall antioxidant acitvity of *Arictum Lappa* was the strongest. Phytochemical analysis of plant extracts indicated the presence of major phytocompounds including Phenolics, Alkaloids, Glycosides, Flavonoids, and Tannins. The tested plant extracts showed promising antioxidant and free radical scavenging activity.

Keywords: medicanl plant, antioxidant activity, free radical scavenging activity, phytocompounds, phenolics

Introduction
Reactive oxygen species (ROS) are an entire class of highly reactive molecules derived from the metabolism of oxygen. ROS, including superoxide radicals, hydroxyl radicals, and hydrogen peroxide, are often generated as byproducts of biological reactions or from exogenous factors. In vivo, some of these ROS play positives roles in cell physiology; however, they may also cause great damage to cell membranes and DNA, including oxidation that causes membrane lipid peroxidation, deceased membrane fluidity, and DNA mutations leading to cancer, degenerative and other disease [1-4].

Mammalian cells posses elaborate defense mechanisms for radical detoxification. Key metabolic steps are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), which destroy toxic peroxides. In addition to antioxidant enzymes, non-enzymatic molecules, including thiore- doxin, thiols, and disulfide-bonding play important roles in antioxidant defense systems. Some of the compounds are of an exogenous nature and are obtained from food, such as α- tocopherol, β-carotene, and ascorbic acid, and such micronutrient elements as Zinc and Selenium [5]. If cellular constituents do no effectively scavenge free radicals, they lead to disease conditions as described above.

Antioxidant-based drugs/formulations for the prevention and treatment of complex disease like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer have appeared during the last 3 decade [6]. This has attracted a great deal of research interest in natural antioxidants. Subsequently, a worldwide trend towards the use of natural phytochemicals present in berry crops, tea, herbs, oilseeds, beans, fruits, and vegetables has increased [7-9].

Several herbs and spices have been reported to exhibit antioxidant, activity, including rosemary, sage, thyme, nutmeg, turmeric, white pepper, chili pepper, ginger, and several Chinese medicinal plant extracts [10-13]. The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanis, courmarins, lignans, catechins, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E. β-carotene, and α- tocopherol are known to possess antioxidant potential [14-16]. A direct relationship between antioxidant activity and phenolic content of plant extracts has been reported [16, 17]. Epidemiological studies have shown that the consumption of foods and beverages rich in phenolic content can reduce the risk of heart disease [18].

Many Indian medicinal plants are considered potential sources of antioxidant compounds. In some cases, their active constituents are known, Terminalia Chebula, T. bellerica, T. muelleri, and Phyllanthus emblica, all of which have antioxidant activity of Hemisdesmus Indicus,

cichoriu intybus, Withania somnifera, Ocimum sanctum, Mangifera indica, and Punica granatum, as determined by several methods, has been poorly documented [19-23].

There ia an increased quest to obtained natural antioxidants with broad-spectrum actions. The minority of the rich diversity of Indian medicinal plants are yet to be scientifically evaluated for such properties. Furthermore, the relationship between phenolic content and antioxidant among the above 12 medicinal plants that are commonly used in the Indian system of medicine ayuunyeda, unani wree selected study. We have previously evaluated these plansts for their broad-spectrum antimicrobial activity (24.25). In this report, we investigated the crude methanolic extracts of these plants for their potential antioxidant activity with the thiobarbituric acid (TBA), ferric thiocyanate (FTC), and 1, 1, biphenyl picryl hydrazyl [DPPH] radical scavenging methods.

Materials and Methods

Preparation of crude plants extracts

Test plants were collected locally or obtained from the Kashmir valley paharoo. All the plants materials were further identified by the department of Botany. University of Kashmir voucher specimens of these plants were deposited in the department of Botany university of Kashmir. About 2000g of dried plant material was soaked in 2.51 of 98% methanol for 8-10 days, stirring every 18 h using a sterile glass rod. The final extract was passed through No.1 whatman filter paper [whatman Ltd., England]. The filtrate obtained was concentrated under vaccum on a rotary evaporator at 40 c and stored at 4 c for further use. The crude extract was obtained by dissolving a known amount of the dry extract in 98% methanol to obtain a stock solution of 40mg ml concentration.

Antioxidant assay

The antioxidant activity of methanol extract was tested using the ferric thiocyanate [FTC] and thiobarbituric acid [TBA] method. The FTC method was used to measure the amount of peroxide at the beginning of lipid peroxidation, in which peroxide will react with ferrous chloride and form ferric ions. Ferric ions will then unite with ammonium thiocyanate and produce ferric thiocyanate. The substance is red, and denser color is indicative of higher absorbance. The TBA method measure free radicals present after peroxide oxidation.

[a] Ferric thiocyanate [FTC] method

The standard method as described by Kikuzaki and Nakatani [12] was used. A mixture of 4.0 mg ofplant extract in 4 ml of absolute ethanol, 4.1 ml of 2.52% linolenic acid in absolute ethanol, 8.0 ml of 0.05 M phosphate buffer [pH 7.0], and 3.9 ml of water was placed in a vial with a screw cap and then placed in a dark were added 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% HCL to the reaction mixture, the absorbance of red color was measured at 500mm every 24 h until one day after the absorbance of control reached its maximum. Butylated hydroxyl toluene [BHT] and a-tocopherol were used as positive controls, while a mixture without a plant sample was used as the negative control.

(b). Thiobarbituric acid (TBA) method

The method of Ottolenghi [26] was followed. Two mili liters of 25% trichloroacetic acid and 2 ml of 0.67% 2-thiobarbituric acid were added to 1 ml of sample solution, prepared with the FTC method. The mixture was placed in a boiling water bath and after cooling was centrifuged at 3000 rpm for 20 min. Absorbance of supernatant was measured at 552 nm. Antioxidant activity was based on the absorbance on the final day of the FTC method.

Free radical scavenging assay

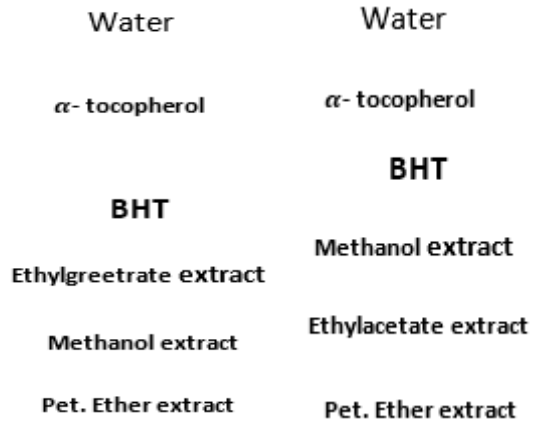
The scavenging activity of DPPH free radicals by different extracts was determined according to the method reported by Gyamfi [27]. Fifty microliters of the plant extract in methanol, yielding 100 μg/ml in each reaction, was mixed with 1 ml of 0.1 mM DPPH in methanol solution and 450 μl of 50 mM Tris HCL buffer (pH 7.4). Methanol (50 μl) only was used as the experimental control. After 30 min of incubation at room temperature, the reduction in the number of DPPH free radicals was measured, reading the absorbance at 517 nm. BHT and α-tocopherol were used as controls. The percent inhibition was calculated from the following equation:

% inhibition = [Absorbance of control – Absorbance of test sample/ Absorbance of control] x 100

Phytochemical analysis

Phytochemical analysis of the major phytoconstituents of the extracts was undertaken using standard qualitative methods (color tests and/or TLC) as described earlier [24].

Total phenolic compound analysis



The amounts of phenolics contents of these extracts were determined with Folin-Ciocalteu reagent using the method of Spanos and Wrolstad [28] as modified by Lister and Wilson [29]. To 50 ml of each sample (3 replicates) 2.5 ml of 10% dilution of Folin-Ciocalteu reagent and 2 ml of Na2CO3 (7.5%, w/v) were added and the resulting mixture was incubated at 45 oC for 15 min. the absorbance of all samples was measured at 765 nm using a Spectronic 20D. Results were expressed as milligrams of Gallic acid equivalent per gram of dry weight (mg GAE/g dw).

Results and discussion

Reactive oxygen species (ROS), from both endogenous and exogenous sources may be involved in the etiologies of such diverse human disease. There is evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs, and medicinal plants [30, 31]. Our attention has been focused in particular plant.

The antioxidant activities of extract were measured by the FTC method and compared with the TBA method at a

concentration of 0.02% in methanolic solutions. The extract tested showed low absorbance values which indicated a high level of antioxidant activity. However, all the methanol extract exhibited strong antioxidant activity as determined by both the FTC and TBA methods, surpassing the activity of the standard commercial antioxidants, alpha-tocopherol, and butylated hydroxyl toluene (Figures 1).

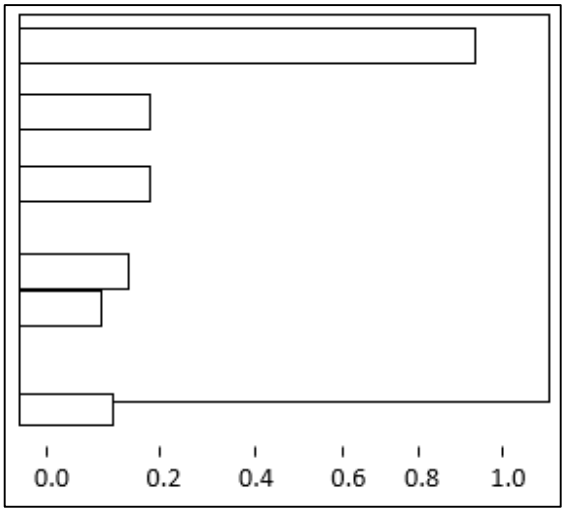
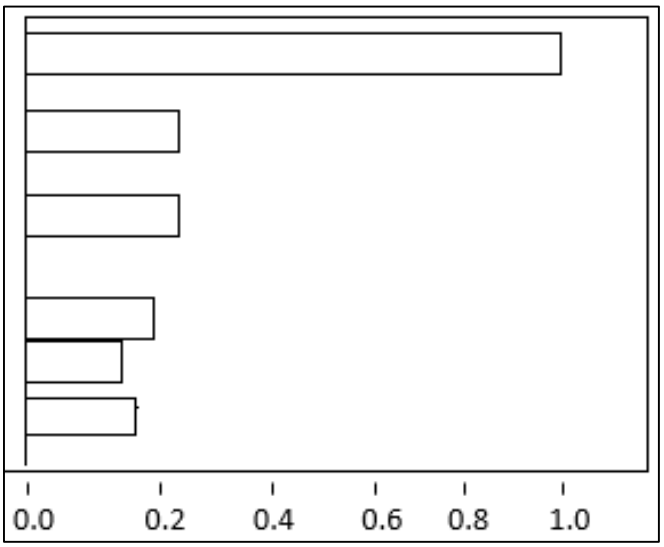


Fig 1: Antioxidant activities of plant extracts determined with the TBA method



Absorbance at 532 nm on the final day of FTC method

Fig 1: Antioxidant activities of plant extracts determined with the TBA Method

The phyto-chemical analysis of the crude extracts indicated the presence of major phyto-compounds including phenolics, alkaloids, glycosides**,** flavonoids, and tannins (Table 1), which may have been responsible for the observed antioxidant activity.

Table 1: Free Radical scavenging activity of Plant extracts determined with the DPPH method and total phenolic contents.

S No.	Extract	Part used	%Decolourization ± SD	*Phyto compounds detected	**Total phenolic contents ± SD
1.	Methanol extract	Leaves	7.97± 1.09	P,T	28.66± 2.52
2.	Ethyl acetate extract	Leaves	69.95± 3.62	G	163.33± 7.37
3.	Pet ether extract	Leaves	73.34± 4.87	A,T	76.83± 8.75

*Phyto-compounds Key: A, Alkaloids, P, Phenols, F, Flavonoids; G, Glycosides, S, Saponins; T, Tannins
**mg/g of dry plant extracts

Medicinal plants are promising sources of potential antioxidants. Further study will be aimed at isolating and identifying the substances responsible for the antioxidant activity of plant extracts, which may be further exploited in herbal formulations.

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