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Study on radical scavenging activity and analysis of bioactive compounds in selected Indian medicinal plants

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ABSTRACT

Antioxidant therapy promises effective role in treatment of diseases caused due to free radicals. The present study is to examine the antioxidant potential of selected medicinal plants namely *Nardostachys jatamansi, Swertia chirayita, Glycyrrhiza glabra, Zingiber officinale, Carum carvi, Trachyspermum ammi, Madhucana indica ,Berberries aristata, Fenniculum vulgare, Myristica fragans.* Ethanolic extract were used for analysis. *B.aristata* showed highest radical scavenging effect on the stable DPPH· radical with IC_{50} (95.9%) followed by *M. fragrans* with IC_{50} (91.8%) which is high in comparison with synthetic antioxidant BHT (74.8%). Superoxide radical scavenging activity, hydroxyl radical scavenging activities, Total antioxidant power, invitro lipid peroxidation were evaluated using concentration range of 1gm,5gm,10gm,15gm,20gm/100ml .Antioxidant activity is reported due to presence of bioactive compounds hence analysed presence of phenols, flavonoids, alkaloids.

Key words: DPPH radicals, in vitro lipid peroxidation, BHT, Phenols.

INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. Free radical role in causing diseases can be compared with role of microorganism in infections disease[1]. Reactive oxygen species are derived from the metabolism of oxygen, this includes superoxide radical (O_2^-), hydroxyl radical (OH^-) and hydrogen peroxide[2,3]. Oxidation process is important in producing free radicals in living system[4]. In cellular oxidation reactions superoxide transformed into other potentially harmful free radicals causing peroxidation of lipid, protein and DNA damage[5].Oxidation process can be augmented by antioxidants defence system by scavenging free radicals giving maximum intracellular protection. Due to depletion of natural antioxidants in different maladies, consumption of antioxidants as free radical scavengers has become necessary, inspite of having synthetic antioxidants[6,7].

Many plant species have been investigated in search for novel antioxidants in the recent time[8,9] but demand still persist .In particular, despite widespread of medicinal plants, the literature supports the importance of medicinal properties of these plants. *N. jatamansi* (Valerianaceae), the dried rhizomes are known as stimulants, bitter tonic[10]. *S.chirayita* (Gentianaceae), stem has antipyretic[11]. *G. glabra* (Leguminosae) roots used to relieve coughs, gastric inflammation[12]. *Z. officinale* (Zingiberacea) roots used as cardiac stimulant[13]. *C.carvi* (Apiaceae) seeds used in stomach disorders to relieve pain, scabies[14]. *T. ammi* (Apiaceace) seeds have platelet aggregation inhibitory action. *M. indica* (Sapotaceae) fruits are used to cure piles, bronchitis. *B.aristata* (Berberidaceae) roots used in piles, pain reliever, antituberculosis. *F.vulgare* (Umbelliferae) seeds are used in bronchitis,. *M.fragans* (Myristicaceae) seeds anti-platelet aggregation, anti-inflammatory activities.

MATERIAL AND METHODS

1, 1, Diphenyl-2-picrylhydrazyl, 2, 4, 6-tripyridyl-s-trizine(TPTZ) purchased from sigma chemicals co. The other chemicals and solvents used in the present study were of analytical grade obtained from local supplier in pure quality .The plant material were purchased from local supplier.

Preparation of plant extract

The plant material were thoroughly cleaned, shade dried and coarse powdered in a mechanical blender. The powder was successively ethanol, by soxhlet extraction method run for 48hrs. Concentration ranging from was prepared (1gm, 5gm, 10gm, 15gm, and 20gm/100ml).

DPPH radical scavenging activity

DPPH scavenging activity was measured by the method of (Cuedet etal, 1997). To 5 ml of a methanolic solution of DPPH (0.004%), 50µl of test extract (1gm, 5gm, 10gm, 15gm, and 20gm/100ml) were added. BHT was used as standard, for control test extract were replaced by ethanol. The reaction mixture were incubated for 30 minutes at 37^{0}C, absorbance was taken at 517nm using Systronic UV-visible spectrophotometer The IC^{50} of inhibition was calculated from following equation A_0-Ax100/ $A_{0.}$ Therefore, A_0 is absorbance of control and A is absorbance of sample. IC_{50} value denotes the concentration of sample required to scavenge 50% of free radicals.

Superoxide radical scavenging activity

The superoxide radical scavenging activity was measured by (Beauchamp and Fedovich 1976) method. Superoxide anion were generated in non-enzymatic hydroxylamine (HA)-EDTA system through the reaction of HA, EDTA and oxygen .It was assayed by reduction of nitroblue tetrazolium .The superoxide anion were generated in reaction mixture containing 1.0ml of sodium carbonate (125mM),0.4ml NBT (24µM) and 0.2 ml of EDTA(0.1mM).The reaction was initiated by adding 0.4ml of hydroxylamine(1mM) and 0.5ml of plant extracts of different concentrations , in control test extract were replaced by ethanol. After 5 minutes of incubation at room temperature, the absorbance was measured at 560nm. The IC_{50} of inhibition was calculated from following equation. A_0-Ax100/$A_{0.}$

Hydroxyl radical scavenging activity

The ability of sample to inhibit hydroxyl radical mediated peroxidation was measured by (Kunchandy and Rao, 1990) with some adaptations. The reaction mixture contained 100µl of plant extracts 500µl of (0.6mM) of deoxyribose in phosphate buffer (20mM, pH 7.4), 500 µl ferric chloride (0.1mM) 500µl EDTA(0.1mM) ,500µl of ascorbic acid (0.1mM) and 100µl of H_2O_2 (1mM) and 800µl of phosphate buffer so that the final volume is 3ml. After incubation for 1hr at 37 ^{0}C add 1.0 ml of TCA(2.8%) and 1.0ml of (thiobarbituric acid) TBA (1%) place the reaction mixture in water bath for 20 minutes at 100 ^{0}C cool and centrifuge if necessary , the absorbance was measured at 532 nm. BHT was used as standard, in control test extract were replaced by ethanol. The IC_{50} of inhibition was calculated from following equation A_0-Ax100/$A_{0.}$

Invitro inhibition of lipid peroxidation

Lipid peroxidation induced by $FeSO_4$ – ascorbate system in sheep liver homogenate by method of (Bishayee and Balasubramaniyam, 1971) and the formed thiobarbituric acid reactive substance (TBARS) was estimated by (Ohkawa etal1979). The liver was obtained from slaughter house collected and washed number of times with normal saline To 0.1ml of sheep liver homogenate in (25%) in Tris-HCl buffer (40mM, pH 7.0 ; KCl (30mM);0.16mM of ferrous ammonium sulphate , 0.06mM ascorbic acid), add 0.4ml of plant extract (1gm,5gm,10gm,15gm,20gm/100ml) and incubate for 1hr at 37 ^{0}C , remove 0.4ml of the mixture and add 0.2 ml of (8.1%) SDS, 1.5 ml of (20%) acetic acid, 1.5ml of (0.8%) TBA and incubate in water bath for 1hr at 92 ^{0}C, cool and add 1mlof distilled water and 5ml of butanol: pyridine (15:1) mixture. Shake the reaction mixture and centrifuge at 4000 rpm for 15 minutes and the absorbance of organic layer was measured at 532 nm.

Total antioxidant by FRAP method

The total antioxidant power was determined by the modified FRAP (ferric chloride reducing ability of plasma) method by (Benzie and Strain method, 1996) In this assay FRAP reagent was prepared by adding 2,4,6-tripyridyl-s-trizine(TPTZ) and ferric chloride forming Fe +3 – TPTZ complex is reduced Fe +2 –TPTZ complex which gives an intense blue colour at 595nm .The

calibration curve was prepared using $FeSO_4$ with concentration ranging from100-1000(M to 1.5ml of FRAP reagent (2, 4, 6-tripyridyl-s-triazine and ferric chloride) add 50(l of plant extracts. The absorbance was measured at 593 nm. The results were expressed as Ascorbic acid Equivalent Antioxidant Capacity (AEAC) in terms of mM.

Analysis of bioactive compounds

Presence of major bioactive compounds were analysed by qualitative test for alkaloids (Hagers test, Mayers test, Wagners test), flavonoids (Pew's,Shinoda test), phenolics (ferric chloride test).

Statistical analysis

The values are expressed as the means + S.D of three determinants.

RESULTS AND DISCUSSION

The etiology of various human diseases as arteriosclerosis, cancer, neurodegenerative

Table 1: DPPH radical scavenging activity with different concentration of ethanolic Extract Comparison of IC_{50} with standard BHT (1mg/ml).The values are expressed as scavenging %. BHT shown 74.8% inhibition

	Total antioxidant activity (DPPH radical % scavenging activity) Concentration of plant extracts in (gm/100ml)				
Plant species	**1**	**5**	**10**	**15**	**20**
N.jatamansi (root)	23.5±0.270	34.6±0.129	60.6±0.571	63.4±0.282	68.7±0.216
S.chirayita (stem)	11.2±0.208	15.1±0.535	24.9±0.369	28.6±0.489	41.4±1.104
G.glabra (roots)	13.4±0.451	32.3±0.294	50.3±0.251	64.8±0.673	68.6±0.707
Z.officinale (root)	10.8±0.129	21.8±0.163	31.4±0.621	37.6±0.683	39.5±0.778
C.carvi (seeds)	10.8±0.115	19.2±0.294	22.1±1.798	29±0.613	38.6±0.976
T.ammi (fruits)	19.2±0.535	41.7±0.355	63.8±1.961	67.8±0.163	75.1±0.244
M. indica (fruits)	16.9±0.182	24.6±0.163	33±0.294	38.7±0.141	69.4±0.941
B. aristata (root)	87.8±0.621	91.8±0.100	92.8±0.163	93±0.208	95.9±0.378
F.vulgare (seeds)	10.2±0.864	14.3±0.294	21±0.331	23.2±0.141	34.2±0.216
M.fragans (seeds)	26.5±0.14	74.4±0.346	87±0.163	90.4±0.420	91.8±0.238

Table 2: Superoxide radical scavenging activity of ethanolic plant extract. Comparison of IC_{50} with standard BHT (1mg/ml), with 69.4% of inhibition

	Total antioxidant activity (superoxide radical scavenging activity %) Concentration of plant extracts in (gm/100ml)				
Plant species	**1**	**5**	**10**	**15**	**20**
N.jatamansi (root)	80.5±0.804	81.8±0.516	86.3±0.244	87.6±0.627	88.3±0.238
S.chirayita (stem)	58±0.288	58.8±0.559	69.7±1.416	70±0.901	81.9±1.515
G.glabra (roots)	63±0.714	65.1±0.697	67±1.084	78±1.322	83.2±3.456
Z.officinale (roots)	58.8±0.282	62.4±1.382	67.4±1.392	71±0.993	75.6±1.886
C.carvi (seeds)	67±1.157	70.2±0.953	74±1.447	77.1±1.842	82.5±0.387
T.ammi (fruits)	34±1.920	38.2±1.271	62.8±2.127	76.5±0.716	83.5±2.525
M. indica (fruits)	74±1.201	79.3±0.758	80.1±0.300	82.5±1.160	83±1.00
B. aristata (root)	82.7±0.057	83.5±0.331	85±2.060	89±0.450	92±0.704
F.vulgare (seeds)	59±1.223	63.8±1.650	66.4±1.195	70±0.704	73±0.732
M.fragans (seeds)	78.6±1.212	81.3±0.443	83.2±0.345	85±1.332	88±0.911

disease has shown the involvement of ROS (4). Evidences say that antioxidants may overcome these deleterious consequences of oxidative stress. Free radical scavenging activity was evaluated by DPPH free radical scavenging method. Ethanol due to high polarity extracts most of the compounds hence exhibit high activity. Increasing concentration of extracts ranging from (1gm, 5gm, 10gm, 15gm, 20gm/100ml) were analysed to find out any proportionality in activity with increase in concentration. The DPPH assay measured hydrogen atom (or one electron) donating activity and hence provided an evaluation of antioxidant activity due to free radical scavenging. Plant extracts showed decrease in absorbency with a stable $DPPH^{\cdot}$ radicals which indicate a high level of radical scavenging activity. Table: 1 it infers that *B.aristata* and *M.fragans* has shown highest radical scavenging activity in dose dependent order *S. chirayita Z.officinale C. carvi F. vulgares* difference in result did not contributed satisfactory activity. Superoxide is biologically important as it can for potent oxidative species, which can damage cellular components . The results are shown in table:-2 gives % of inhibition which is concentration dependent and *N.jatamansi, M.fragans* showed almost similar

Table 3: Gives IC_{50} value which is concentration dependent and compared with BHT (1mg/ml) standard which exhibited 71% scavenging activity

	Total antioxidant activity (superoxide radical scavenging activity %) Concentration of plant extracts in (gm/100ml)				
Plant species	**1**	**5**	**10**	**15**	**20**
N.jatamansi (root)	60.4±2.219	70.5±1.212	77.8±2.737	80±0.818	83±1.388
S. chirayita (stem)	56.3±8.9	60.4±4.830	62.9±1.512	64.3±1.247	71.2±1.160
G.glabra (roots)	55±1.07	60.9±1.122	62.6±3.746	63±1.414	66±1.214
Z.officinale (roots)	55.2±1.258	59±1.474	61±1.814	64±1.453	69±1.247
C.carvi (seeds)	39±2.606	41.2±2.29	46.4±0.663	47.2±1.402	49±1.390
T.ammi (fruits)	58±3.23	56±1.164	54.6±0.896	60.1±1.512	60.4±0.568
M. indica (fruits)	56.5±2.11	68.3±0.743	71.5±2.273	72±1.694	76.2±0.848
B. aristata (root)	71.6±2.129	83±1.388	93.6±0.945	96.5±1.298	97±0.816
F.vulgare (seeds)	51.4±4.867	59±1.388	61.5±0.894	66±1.515	72.3±0.943
M.fragans (seeds)	70.5±2.124	78.1±2.843	82.2±1.357	86.6±1.248	89.9±1.555

Table 4: In vitro lipid peroxidation of ethanolic extract in comparison with standard BHT (1mg/ml) 79.3% of inhibition

	Total antioxidant activity (superoxide radical scavenging activity %) Concentration of plant extracts in (gm/100ml)				
Plant species	**1**	**5**	**10**	**15**	**20**
N.jatamansi (roots)	84.2±0.572	88.5±0.529	93.5±0.920	94.4±0.420	96.3±0.454
S. chirayita (stem)	85.6±1.351	87±0.529	90.8±0.840	92.6±0.288	94.9±0.141
G.glabra (roots)	82.6±0.476	88±0.051	92.3±0.244	93.3±0.848	96.3±0.270
Z.officinale (roots)	85.4±0.420	89.6±0.282	92.3±0.820	93.8±0.624	95.9±0.476
C.carvi (seeds)	80.3±0.3	84±0.387	90.3±0.496	92.7±0.979	93.5±0.369
T.ammi (fruits)	70.5±0.5	87.8±0.238	91.7±0.559	92.5±0.412	94.1±0.141
M. indica (fruits)	70.5±0.5	87.8±0.238	91.7±0.559	92.5±0.412	94.1±0.141
B. aristata (root)	90±0.05	92±0.932	93.5±0.5	97.5±0.705	98±0.052
F.vulgare (seeds)	86.5±0.288	90±0.387	94.5±0.605	95±0.05	97.7±0.270
M.fragans (seeds)	61.4±0.516	89±0.866	92±0.05	94.6±0.420	96.3±1.852

pattern highest activity shown by *B.aristata* BHT (1mg/ml) a synthetic antioxidant used as standard for comparision Hydroxyl radical is very reactive and can be generated in biological cells through Fenton reaction radical scavenging activity. Table 3 showed 97% Radical scavenging by *B.aristata* which is significantly high. Initiation of lipid peroxidation by ferrous takes place through hydroxyl radical by Fenton reaction. The degree of inhibition of $FeSO_4$ induced lipid peroxidation in sheep liver homogenate in a function of dose dependent. The inhibition could be due to scavenging hydroxyl radical or superoxide radical or by chelating iron Hydroxyl radicals can interact with membrane lipids to form lipid hydroperoxides (Valentao *et al.,* 2002). This can lead to production of alkoxy and peroxy radicals causing DNA damage (Reimersma *et al.,* 2000) Table 4 gives data regarding Inhibition of lipid peroxidation which was significant with all plant extracts.

It has been recognized that most of the plants considered in the present study showed significant activity when compared with synthetic antioxidant like BHT among these *B.aristata* ,*M.fragans, N.jatamansi* were proved to be most promising source of antioxidant and can be used

Table 5: Total antioxidants activity by FRAP methodwith different concentration of ethanolic extract (concentration in mM)

Plant species	Total antioxidant activity (superoxide radical scavenging activity %) Concentration of plant extracts in (gm/100ml) 1	5	10	15	20
N.jatamansi (root)	0.116±0.008	0.25±0.005	0.49±0.005	0.49±0.005	0.58±0.005
S. chirayita (stem)	0.07±0.008	0.11±0.005	0.19±0.008	0.20±0.005	0.22±0.093
G.glabra (roots)	0.118±0.002	0.26±0.008	0.49±0.005	0.52±0.005	0.62±0.014
Z.officinale (roots)	0.90±0.003	0.11±0.005	0.28±0.008	0.13±0.008	0.16±0.008
C.carvi (seeds)	0.08±0.001	0.10±0.008	0.13±0.005	0.18±0.005	0.25±0.01
T.ammi (fruits)	0.10±0.005	0.22±0.005	0.48±0.012	0.89±0.020	2.7±0.05
M. indica (fruits)	0.13±0.005	0.17±0.008	0.25±0.012	1.0 ±0.042	2.3±0.05
B. aristata (root)	0.93±0.005	1.9±0.001	5.0 ±0.08	8.9±0.14	10.7±0.20
F.vulgare (seeds)	0.03±0.008	0.06±0.008	0.07±0.012	0.13±0.005	0.15±0.005
M.fragans (seeds)	0.18±0.001	0.66±0.012	0.8±0.001	2.1±0.05	2.6±0.346

Table 6: Qualitative phytochemical analyses for the presence of alkaloids, Flavonoids and Phenolics

Plants species	Bioactive compounds		
	Alkaloids	Flavonoids	Phenolics
N.jatamansi (root)	+	+	+
S.chirayita (stem)	+	+	+
G.glabra (roots)	-	+	+
Z.officinale (roots)	+	+	-
C.carvi (seeds)	-	+	-
T.ammi (fruits)	+	-	-
M. indica (fruits)	-	+	-
B. aristata (root)	+	+	+
F.vulgare (seeds)	+	-	-
M.fragans (seeds)	+	+	+

as natural future source of antioxidants .Hence studies has to done to analyze bioactive responsible for radical scavenging activity In long term study on these plant species may be valuable in treatment for free radical induced damage.

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