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ANTI-OXIDANT ACTIVITY OF SELECTED MEDICINAL PLANTS OF THE HIMALAYAN REGION

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ABSTRACT: Natural medicines and nutraceuticals are good sources of anti-oxidant and are gaining popularity for their capacity to enhance, health-protective effects. The aim of the present study was to investigate the anti-oxidant activity of some natural medicine from the Himalayan region of Nepal. Carefully collected plant materials were subjected to maceration with methanol for about 48 hours. Anti-oxidant activity of methanolic extracts was assessed using DPPH as a free radical in different concentrations (0. 1 µg/ml, 1 µg/ml, 10µg/ml, 100µg/ml). Percent inhibition and effective Concentration (EC_{50}) values were calculated. EC_{50} of *Acacia catechu* (0.89 µg/ml), *Cissus repens* (3.44µg/ml) *Psidium guajava* (3.65µg/ml) were calculated and found to be more effective than ascorbic acid (4.48µg/ml). *Drynaria propinque* (5.21µg/ml) *Eskemukerjea megacarpum* (5.24µg/ml) and *Nelumbo nucifera* (6.76µg/ml) were comparable to standard anti-oxidants whereas *Ficus relifiosa*, *Tinospora sinensis*, *Elaeocarpus sphaericus*, and *Cannavis sativa* showed poor free radical-scavenging activity. Our results revealed that *Acacia catechu* (resin) showed greater anti-oxidant activity than other extracts and standard, suggesting that it could be the better anti-oxidant and diseases preventive remedies and should be developed as a future pharmaceutical or nutraceutical.

INTRODUCTION: 250,000 to 500,000 species of plants which are estimated that are known to man. Out of these species, only 10% are used for medicinal purposes [1, 2]. Nepal is a Himalayan country that has a diversity of medicinal plants (~7000 species). Among them, only 700 species are documented as defined medicinal use but still Nepalese people are incapable to consume these resources accurately [3]. In our study, most of the selected crude drugs are practices by Baidhyas (Traditional healers) in different region of Nepal. To investigate the anti-oxidant properties, the following medicinal plants have been selected.

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Medicinal plants contains diverse groups of chemical constituents and micronutrients viz. flavonoids, phenolic acids, phenolic diterpenes, tannins, vitamin C and E that can be found in various parts of the several plant species and vegetables we take [4].

We are familiar with frequently generated free radicals and other reactive species that are consequence of cellular metabolism and are accumulated in the cells and tissues that finally causes the oxidative stress. Many cellular system developed resistance against ROS mediated oxidative stress by scavenging free radicals and preventing from lipid peroxidation which ultimately protect from several chronic disorders including neuropsychiatric, cancer, epilepsy, inflammatory damage, atherosclerosis, stroke, diabetes, neurodegenerative, rheumatoid arthritis and aging. Several cellular (superoxide dismutase, glutathione, GSH peroxidases, GSH reductase and

catalase) natural (Vitamin E/C and many polyphenols) and synthetic antioxidants (BHT) counteract the deleterious effect of ROS/RNS by donating their own electrons [5-7].

TABLE 1. LIST OF THE COLLECTED MEDICINAL PLANTS.

S.N	Biological source	Common Name	Parts used
1.	*Eskemukerjea megacarpum*	Bhotekhair	Underground
2.	*Acacia catechu*	Khair	Resins
3.	*Cissus repens*	Jogeelahara	Climber
4.	*Psidium guajava*	Amba	Leaves
5.	*Ficus religiosa*	Peepal	Fruits
6.	*Tinospora sinensis*	Gujro	Climber
7.	*Drynaria propinque*	Kammari	Rhizomes
8.	*Elaeocarpus sphaericus*	Rudrakshya	Fruits
9.	*Nelumbo nucifera*	Kamal	Seeds
10.	*Cannavis sativa*	Bhang	Fruits

MATERIALS AND METHOD:

Collection of Natural product

Traditionally used selected above medicinal crude drugs were collected from the different region of the country. The authentic samples of these natural remedies were preserved in the museum of Material Medica, The School of Health and Allied Science, Department of Pharmaceutical Science, Pokhara University.

Chemicals and Equipments

HPLC grade methanol used for the DPPH assay was supplied from Merck Limited India. Ascorbic acid was the product of Qualigens Fine Chemicals, India.1, 1-diphenyl-2-picryl hydrazyl (DPPH) was obtained from Wako Pure Chemicals Co. Ltd. Osaka, Japan.

Preparation of Extracts

Collected plants were carefully washed with distilled water and dried. Each dried medicinal plants (100g) were grounded so as to get coarse particle size.

The course crude part of the plants were allowed for the maceration with methanol (500ml) at room temperature for 48 hours and filtered. The residue obtained is again extracted with 500ml of methanol in same condition and time.

DPPH Radical Scavenging Activity

Preparation of DPPH Solution

11.82 mg of DPPH (MW: 394.32g/mol; Density; 1.40 g/cm3) was dissolved in 500mL of methanol in order to prepare 60µM DPPH solution.

Determination of DPPH Radical Scavenging Activity

1, 1 – diphenyl – 2 - picryl hydrazyl (DPPH) scavenging activity was carried out according to slight modification of blois et al [8]. DPPH is a relatively stable free radical, 2 ml of methanolic solution of each extract at various concentration (0. 1 µg/ml, 1µg/ml, 10µg/ml, 100µg/ml) were mixed with 2 ml of methanolic solution of DPPH (60µM). After vortex mixing, the mixture was incubated for 30 minutes at room temperature and the absorbance values were measured at 517 nm.

Reactive oxygen activity or radical scavenging activity of medicinal extracts was calculated by using following equation:

% Inhibition = (absorbance of control - absorbance of sample)/absorbance of control] X100.

Where, control is the solvent (methanol) without extract. Ascorbic acid was used as the positive control.

From these data, calibration curve were plotted and Effective Concentration (EC_{50}) values were calculated.

Stastistical Analysis

All the values and data were expressed as Mean±SEM, N=3, from Linear regression method.

RESULTS:

EC_{50} of three plants viz *A. catechu* (0.89 µg/ml), *C. repens* (3.44 µg/ml) *P. Guajava* (3.65 µg/ml) were less than Ascorbic acid (4.48 µg/ml) which indicate that these medicinal plants are more potent than standard drug where in *D. propinque* (5.21 µg/ml) *E. megacarpum* (5.24 µg/ml) and *N. nucifera* (6.76 µg/ml) were comparable to standard anti-oxidant. Remaining crude extracts does not showed satisfactory anti-oxidant activity.

TABLE.2: PERCENTAGE INHIBITION AND EFFECTIVE CONCENTRATION (50) VALUE OF SELECTED MEDICINAL PLANTS AND STANDARD ASCORBIC ACID AGAINST DPPH FREE RADICALS.

		% Inhibition				
S.No	**Sample**	**0.1 µg/ml**	**1 µg/ml**	**10 µg/ml**	**100 µg/ml**	**EC_{50}**
1	Ascorbic acid	6.81±2.02	25.43±1.12	88.96±1.53	93.93±0.10	4.48
2	*A. catechu*	29.54±1.84	52.70±1.68	85.60±1.73	88.74±1.91	0.89
3	*C. repens*	27.70±1.19	32.46±1.97	87.44±2.65	91.45±0.65	3.44
4	*P. Guajava*	21.42±0.32	35.82±1.02	88.41±0.28	90.47±0.38	3.65
5	*D. propinque*	20.34±1.50	24.89±1.02	78.46±3.58	87.87±0.28	5.21
6	*E. megacarpum*	16.23±1.20	16.23±3.84	87.77±0.38	87.12±0.42	5.24
7	*N. nucifera*	5.41±1.42	8.65±2.65	73.16±1.73	86.90±0.10	6.76
8	*F. religiosa*	29.22±0.36	33.33±0.21	34.63±0.10	80.41±0.46	40.21
9	*T. sinensis*	23.80±0.57	27.70±0.28	35.82±0.10	74.67±4.22	42.84
10	*C. sativa*	1.94±0.32	5.19±1.04	18.29±4.13	87.66±0.67	51.13
11	*E. sphaericus*	23.26±1.24	23.26±2.02	36.14±3.36	92.85±0.49	31.98

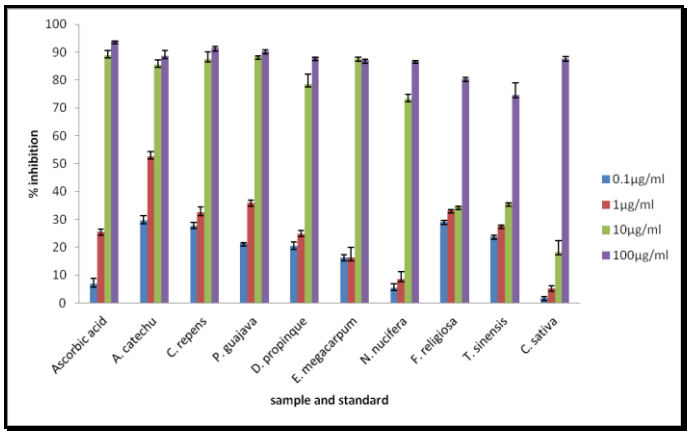

FIG 1. % INHIBITION OF DIFFERENT PLANT EXTRACTS AND ASCORBIC ACID.

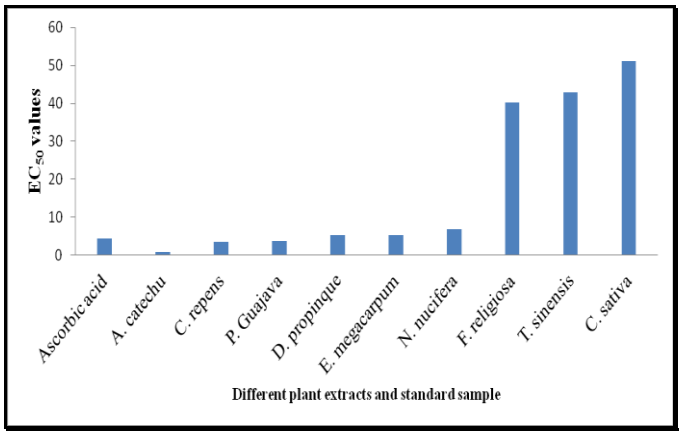

FIG 2. EFFECTIVE CONCENTRATION (50) OF SELECTED MEDICINAL PLANTS AND STANDARD ANTIOXIDANT.

DISCUSSIONS: The present study was intended to examine the anti-oxidant activity of selected medicinal plants of Himalayan regions of Nepal which are still practices in different community as a primary health care system. Selected medicinal plants were assessed by DPPH technique. This method is easy, simple, sensitive, reproducible and cheap. DPPH is the famous, stable, proton free radical due to the delocalization of the unused (spare) electrons. This delocalization of electrons is responsible for the deep violet color of DPPH with a characteristics absorption band in methanol.

Anti-oxidants are those compounds which can donate hydrogen atom to the free radicals, on addition of hydrogen atom, the free radicals will reduced to neutral compounds with loss of violet color [9 -12].Plants contains several secondary metabolites, the phenolic components (flavonoids, tannins, phenolic acid and diterpene), vitamin E and C [4]. Anti-oxidant are the secondary metabolites of a plant which on small quantity scavenges the free radicals and prevent several chronic disease by donating their own electrons to ROS/RNS [10, 6].

Traditionally *E. megacarpum* is used for an extreme bleeding control during menstruation. It contains Gallic acid, Astringin, 5,7-dihydroxycoumarin, Trans feruloyltyramine, Beta-Glucogallin, Trifolin, (-)Epigallocatechin3-0-gallate hyperine, Myricetin 3-0-β-D-galactopyranoside, Myricitrin, Reveratorol, Astrigenin, Piceid, Resveratorol 3-0-β-D(6-0-galloyl) glucopyranoside, Catechin (4α 8)-epigallocatechin 3-0-gallate, idaein, and Epicatechin 3-0- gallate-(4β 8)-epigallocatechin 3-0-gallate [13]. *C. repens is* applied in boils and abscesses for maturation. It is useful in sloughing and foetid ulcers [14]. Useful in cough and bronchitis[15].

D. propinque contains Propinqualin, whose structure was recognized as (-)-epiafzelechin-3-O-beta-D-allopyranoside. The other three were 4-O-beta-D-glucopyranosyl caffeic acid, beta-sitosterol-3-O-beta-D-glucopyranoside and sucrose and it is traditionally used as analgesic and anti-

inflammatory for bachache and rheumatoid arthritis[14].

T. sinensis is used in chronic diarrhoea, chronic dysentery, cough, stomachache, urinary troubles and diuretic. The plant is commonly used in rheumatism and jaundice. The plant enriches the blood[16, 3]. It contains alkaloids, phenolic compounds, tannins and flavonoids [17].

Leaves of *P. guajava* are astringent for bowels, wounds and ulcer; their decoction is used in cholera*A.* and for striking the vomiting and diarrhea. It contains Amritoside, arjunoleic acid, guavin A, B, C and D, Guijaverin, 3, 4 hexahydroxydiphenoylarabinose and isostrctinin [14]. Seeds of *N. nucifera* contain huge amount of glutathione and several alkaloids such as liensinine and isoliensinine and neferine[18]. It also contains linoleic, linolenic, palmitic, oleic acid and myristic acid [14].It is used for cooling down the burning sensation, used in piles as demulsant and applied in ringworms, dysentery and dyspepsia [3, 14].

Fruits of *F. religiosa* is used as cooling and as laxatives in asthma and as digestives. Chemical constituents of fruit of *F. religiosa* are asgaragine, tyrosine, undecane, tridecane, tetradecane, (e)-β-ocimene, α-thujene, α-pinene, β-pinene, α-terpinene, limonene, dendrolasine, dendrolasine α-ylangene, α-copaene, β-bourbonene, β-caryophyllene, α-trans bergamotene, aromadendrene, α-humulene, alloaromadendrene, germacrene, bicyclogermacrene, γ-cadinene and δ-cadinene [19].

Fruits of *E. sphaericus* are used as liver tonic and is useful in the disease of head, epileptic fits and mental disorder[14]. It contains indolizidine type of Alkaloids several minerals, vitamins, steroids and flavanoids [20].

Seeds of *C. sativa* are used as carminative and diuretics and in ear disease, and contain essential oils [21].

In our study out of tenth selected plants, three plants *A. catechu*, *C. repens* and *P. Guajava* showed better antioxidant activity and three plants such as *D. propinque*, *N. nucifera* and *E. megacarpum* exhibited similar to standard drugs ascorbic acid. Not extensive research has been done in resin part of the *A. catechu*. Resin/gums parts of *A. catechu* showed highest antioxidant activity which contains mainly epicatechin and quercetin as an active ingreadients.

Traditionally the resin part of the plants are used in cough and sore throat [22].

The order of anti-oxidant activities are as follows: *catechu*> *C. repens*> *P. Guajava*> Ascorbic acid> *D. propinque*> *E. megacarpum*> *N. nucifera*> *E. sphaericus*> *F. religiosa*> *T. sinensis*> *C. sativa.* Free radicals scavenging or anti-oxidant activity may be the possible mechanism which is useful in traditional medicine for diversity of disorder.

CONCLUSIONS: Our study reports that resin of *A. catechu* exhibited several folded potent anti-oxidant activity than standard ascorbic acid. It was revealed for the first time that resin part of the *A. catechu* exhibited outstanding anti-oxidant activity by DPPH method. Amongst ten medicinal plant extracts, six crude extracts were good anti-oxidant and probably the better future formulation for the prevention and treatments of various ailments arising from oxidative stress like cardiovascular, neurodegenerative, stroke, autoimmune diseases, aging and cancer [23, 24].Further research on this plants regarding in vivo experiment should be made.

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