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IN VITRO ANTIOXIDANT ACTIVITY OF *ABUTILON INDICUM* LINN LEAVES: EFFECT OF EXTRACTION SOLVENTS

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ABSTRACT

In vitro antioxidant activity of *Abutilon indicum* Linn (*A. indicum* L.) is known in literature. But there is controversy on the solvent used for extraction by which maximum antioxidant activity can be obtained. Aim of the present study was, therefore, to see the effect of different extraction solvents on in vitro antioxidant activity of *A. indicum* L. leaves. *A. indicum* L. leaves were collected from the medicinal plant garden of the North Bengal University and identified by the taxonomist. Solvent extractions of the leaves were made separately by using chloroform, petroleum ether, methanol, ethanol, ethyl acetate and acetone. Extracts were separately dried and processed for *in vitro* antioxidant activity through superoxide anion generation by xanthine-/xanthine oxidase assay, linoleic acid peroxidation assay and by DPPH photometric assay. Quercetin was used as standard antioxidant. Results showed that ethanol extract of *A. indicum* L. leaves had maximum antioxidant activity against the methods used which was comparable to that of quercetin.

KEYWORDS: *Abutilon indicum* linn. Leaves, solvent extractions, xanthine-/xanthine oxidase assay, linoleic acid peroxidation assay, DPPH photometric assay.

1. INTRODUCTION

*A. indicum (*Family: *Malvaceae),* commonly known as Abutilon, Indian mallow is found in Sri Lanka, topical regions of America and Malaysia. It is also found as a weed in sub-Himalayan tracts, hills up to 1200 m and in hotter parts of India. The plant is a perennial shrub, softly tomentose and up to 3 m in height. Stems are stout and branched, root is cylindrical, flowers are yellow or orange-yellow. Leaves are evergreen, stipulate, and cordate.

Fruits are capsule, densely pubescent. The bark is flattened and the seeds are minutely stellate-hairy, black or dark brown.[1-2]

The plant has several traditional uses. Whole plant is used as a febrifuge, anthelminitic. It is also used in gout, ulcers, inflammation and in treatment of urinary tract problems. Seeds are used in piles as laxative. Bark is given in strangury and urinary complaints and is valued as a diuretic. Decoction of leaves is used in toothache, catarrhal bilious, bronchitis, diarrhoea, gonorrhoea and inflammation of bladder as well as in fever. Infusion of root is useful in fever as a cooling medicine, heamatouria, strangury and also in leprosy. Root is also used as a pulmonary sedative and diuretic. In Chinese medicine seeds are used as an emollient and demulcent. In Unani systems of medicine the plant is used in chest troubles, piles, bronchitis and gonorrhea. Folk practitioners use this plant as mouthwash, for curing allergy, blood dysentery and fever.[3]

Many bioactive compounds have isolated from different parts of the plant. Whole plant contains caffeic acid, fumaric acid, *β*-sitosterol, vanillic acid, *p*-coumaric acid, abutilon A, (R)-N-(1'-methoxycarbonyl-2'phenylethyl)-4-hydroxybenzamide, phydroxybenzoic, galacturonic, *p*-β-D-glycosyloxybenzoic etc. Oil of the plant consists of farnesol, borenol, geraniol, geranyl acetate, elemene and α-cineole. Aerial Part contains vanillic acid, caffeic acid, p-hydroxybenzoic acid, β – sitosterol, fumaric acid, p-coumarin, p - β – Dglucosyloxybenzoic acids, gluco-vanilloyl glucose, amino acids like threonine, serine, leucine, aspartic acid, histidine etc. Leaves contain terpenes, hydrocarbon, flavonoids, amino acids, ketone, aldehyde, fatty acids like stearic, palmitic linoleic, oleic etc. Root contains endesmol, α-pinene, caryophyllene, caryophyllene oxide. Flower contains flavonoids like apigenin 7-O-beta-glucopyranoside, quercetin 3-O-beta-glucopyranoside, luteolin, chrysoeriol, luteolin 7-O-beta glucopyranoside, chrysoeriol 7-O-betaglucopyranoside, quercetin 3-Oalpha- rhamnopyranosyl (1 --> 6)-beta-glucopyranoside. Fruits contain flavonoids and alkaloids Seed contains water soluble galactomannan, *cis* 12, 13-epoxyoleic (vernolic) acid, 9, 10- methylene octadec-9-enoic (sterculic) acid, as well as 8, 9- methylene-heptadec-8-enoic (malvalic) acid.[4-5]

A.indicum has several pharmacological activities like anti-oxidant, anti-microbial, anti-fertility, anti-cancer, anti-diarrhoeal, anti-convulsant, anti- asthmatic, anti –ulcer, anti-bacterial, anti- inflammatory, anti-proliferative, anti-arthritic, anti-diabetic, anti-pyretic, anti-malarial, anti-estrogenic, hepatoprotective, hypoglycemic and wound healing. Further, the

plant showed analgesic and sedative property as well as larvicidal – diuretic - immunomodulatory activity.[6-7]

Antioxidant activity of *A. indicum* L. is reported in literature but there are different opinions on the use of extraction solvent by which maximum activity may be obtained.[8-14] Therefore, aim of the present work was to see effect of extraction solvents on in vitro antioxidant activity of *A. indicum* L. leaves.

2. METHODOLOGY

2.1 Collection of plant materials

A. indicum L. leaves were collected in morning hours (9 – 10 AM) from the medicinal plants garden of the University of North Bengal, Siliguri ($26^0 41' 30.9984"$ N, $88^0 27' 4.5756"$ E, elevation, 410 ft), Dist. Darjeeling, West Bengal, sometimes in the month of July – August 2016. Leaves were authenticated by the taxonomist of the department of Botany of the said University. A voucher specimen (No. SM-MB-012/19) was kept in the department of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences of Sikkim Manipal University, Gangtok, Sikkim, India for future references.

***Abutilon indicum* Linn.**

2.2 Preparation of plant materials

Leaves of *A. indicum* L. were washed thoroughly, shed dried and powered. The powder, used as test drug, was stored desiccated at 4 ^{0}C until further use.

2.3 Solvent extraction

Test drug (100 g) was extracted separately with 500 ml of using chloroform, petroleum ether, methanol, ethanol, ethyl acetate and acetone in soxhlet at 37^0C for 15 minutes. The extract was filtered and the filtrate was evaporated to dryness *in vacuo* with rotary evaporator at 40 – 50 ^{0}C. This was applied separately for all extracts. Brown masses obtained were used for *in vitro* antioxidant assay.

2.4 Antioxidant assays

In vitro anti oxidant activity of different extracts of *A. indicum* L. leaves was assayed through superoxide anion generation by xanthine-/xanthine oxidase assay[15], linoleic acid peroxidation assay[16] and by DPPH photometric assay.[17]

2.5 Chemicals

Chemicals required for the study were purchased from Meerck, Loba Chem. Lab and Himedia Lab.

2.6 Statistical analysis

The statistical significance between antioxidant activity values of the powdered leaves of *A. indicum* L. was evaluated with a Duncan's multiple range test (DMRT). 5% was considered to be statistically significant.[18]

3. RESULTS

In vitro antioxidant activity of powders obtained from different extracts of *A. indicum* L. through superoxide anion generation by xanthine-/xanthine oxidase assay, linoleic acid peroxidation assay and by DPPH photometric assay were given in Table. 1.

It appears from the table that powder obtained from different solvent extractions of *A. indicum* L. leaves had in vitro antioxidant activity but maximum activity was found in the ethanol extract followed by ethyl acetate extract. Inhibitions in xanthine oxidase, linoleic acid peroxidation and DPPH in ethanol extract were found 92.7%, 91.4% and 96.7% respectively. Results were comparable with quercetin where inhibitions in xanthine oxidase, linoleic acid and DPPH came 100%, 85.4% and 96.4% respectively.

Table 1: Inhibitory activity of xanthine oxidation and linoleic acid peroxidation and scavenging capacity of DPPH by the powder obtained from different solvent extracts of *A. indicum* L. leaves.

Powder obtained from extract of *A. indicum* L. leaves	Xanthine oxidase (% inhibition)	Linoleic acid peroxidation (% inhibition)	DPPH (% inhibition)
Petroleum ether	11.2 ± 0.6	16.2 ± 0.6	14.4 ± 0.3
Methanol	24.1 ± 0.8	27.5 ± 0.6	28.7 ± 0.5
Ethyl acetate	40.4 ± 1.2	39.6 ± 1.1	38.3 ± 0.9
Ethanol	92.7 ± 1.2*	91.4 ± 1.0*	96.7 ±1.1 *
Acetone	38.6 ± 1.1	35.1 ± 0.6	33.5 ± 0.9
Chloroform	34.5 ± 0.9	29.6 ± 0.5	27.2 ± 0.6
Quercetin	100 ± 0.02	85.4 ± 0.4	96.4 ± 0.4

Concentration used: 100 µg / ml. Dose was as per our earlier report.[19] Results were a mean of triplicate experiments ± SD. *Significant

4. DISCUSSION

Plants possessing anti oxidant activity are known in literature. It is also known that anti oxidant activity of a plant may vary with nature of extraction solvents.[20] Dent *et al.* used ethanol, methanol and acetone as extraction solvents of dry sage leaves and observed that ethanol extract had maximum antioxidant activity.[21] Quasim *et al.* studied effect of extraction solvents on polyphenols and antioxidant activity of medicinal halophytes and noted that aqueous methanol extracts of coastal halophytes had comparatively higher antioxidant activity.[22] Using methanol, ethyl acetate, acetone, ethanol, and hexane as extraction solvents for walnut green husk, it was observed that acetone, ethanol, and methanol extracts exhibited stronger antioxidant activities, followed by ethyl-acetate and the lowest was for hexane extract.[23] Thouri et al. carried out effect of solvents extraction on phytochemical components and biological activities of Tunisian date seeds. (var. Korkobbi and Arechti). Result showed that the polar solvent exhibited the highest amount of bioactive compounds specially polyphenols which are responsible for the antioxidant activity.[24]

While studying antioxidant activity of *A. indicum* L. different workers had different observations. Chakraborthy, Srikanth *et al.,* Ahmad and Khan, Evanjaline and Mohan observed that methanol extract of *A. indicum* L. had maximum antioxidant activity.[8-11] Soundaryadevi and Jeyamanikandan noted that acetone extract of *A. indicum* L. had maximum antioxidant activity.[12] While Yasmin Yasmin *et al.* showed butanol extract of *A.*

indicum L. had maximum antioxidant activity[13], Kaushik *et al.* demonstrated that ethanol extract of *A. indicum* L. had maximum antioxidant activity.[14]

In the present study solvents like chloroform, petroleum ether, methanol, ethanol, ethyl acetate and acetone were used as extraction solvents of *A. indicum* L. Ethanol extract showed maximum in vitro antioxidant activity as revealed by maximum inhibition of xanthine oxidation and linoleic acid peroxidation as well as scavenging capacity of DPPH in comparison to that of other solvent extracts (Figure – 1). This study, therefore, is in agreement with the observation of Kaushik *et al.*[14]

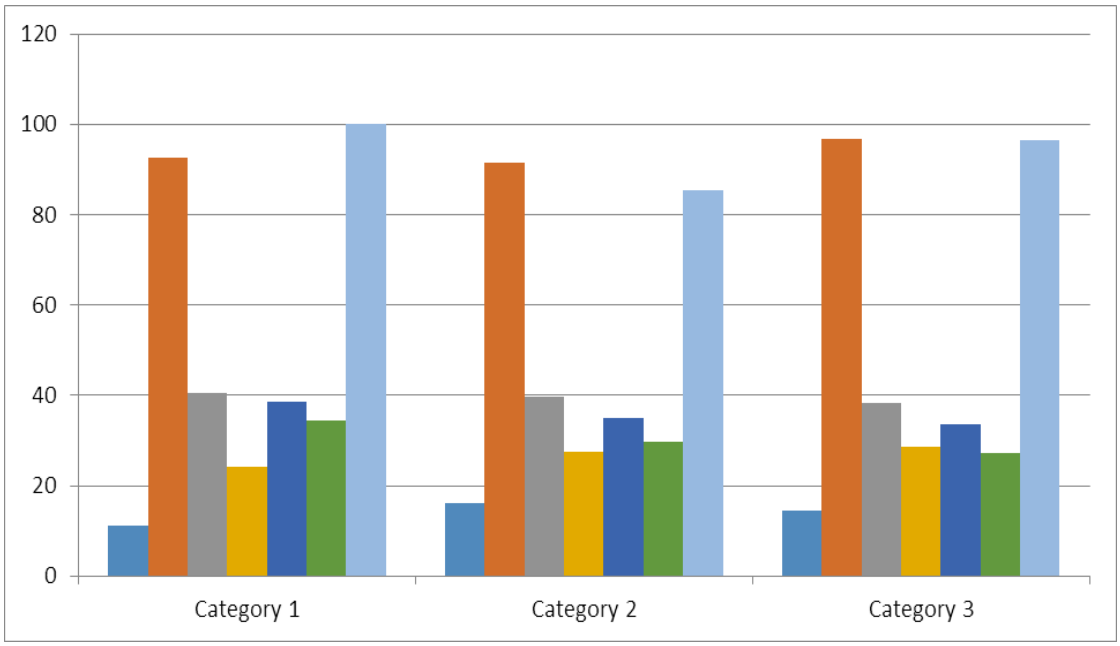

Figure 1: Showing % inhibition of xanthine oxidation, linoleic acid peroxidation and scavenging capacity of DPPH by different solvent extract of *A. indicum* L. leaves.

Category 1: Xanthine oxidase (% inhibition) Category 2: Linoleic acid peroxidation (% inhibition) Category 3: DPPH (% inhibition)

■ Petroleum ether ■ Ethanol ■ Ethyl acetate ■ Methanol ■ Acetone ■ Chloroform ■ Quercetin

Secondary metabolites of plants are usually responsible for their biological activities. It is known that season has significant effect on synthesis of secondary metabolite in plants thereby changing their biologic activity.[25-27] It is, therefore, worth to investigate the seasonal effect on in vitro antioxidant activity of *A. indicum* L. leaves. Work is this direction is now in progress in our laboratory.

CONCLUSION

Toxicity of synthetic antioxidants like butylated hydroxyanisole and butylated hydroxytoluene is known and considered a matter of concern. They may even develop cancer. Therefore, there are high demands for naturally occurring antioxidants. As in present study methanol extract of *A. indicum* L. leaves powder showed maximum in vitro antioxidant activity, *A. indicum* L. leaves may be further investigated for search of natural antioxidant compounds.

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Conflict of interest: Nil.

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