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Comparative evaluation for in vitro Antioxidant activity from *Artocarpus heterophyllus* Lamk and *Balanites aegyptiaca* L.

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Abstract: Present investigation deals with evaluation of in vitro antioxidant activity of ***Balanites aegyptiaca*** and ***Artocarpous heterophyllus*** using DPPH method. Highest antioxidant activity of 42.14 % was reported for aqueous extract of *Balanites* compared to acetone extract with inhibition 13.22 %. Lowest antioxidant activity 12.34 % was noted for acetone extract from the seed of *Artocarpus* whereas, chloroform extract of the same showed 14.87% inhibition. Reducing power increases with increase concentration of the each extract from 0.02 to 0.1 mg/ml. Preliminary phytochemical screening revealed presence of tannins, terpenoid, saponins, anthraquinones. Presence of saponins in the extracts possessing high antioxidant activity and its absence in other tested extracts indicates a possible role for this secondary metabolite for the tested activity. Results obtained in the present study, indicate aqueous leaves extract of *Balanites* as a potential source for natural antioxidant among the four different tested extracts.

Keywords- Antioxidant activity, DPPH method, Balanites , artocarpous, Phytochemicals.

Introduction.

Free radicals are chemically active products of metabolism and include reactive oxygen species (ROS), in its variety of forms, namely superoxide anion ($O2^{\bullet-}$), hydroxyl ($HO^{\bullet}$), and peroxyl ($ROO^{\bullet}$) radicals, or reactive nitrogen species (RNS), which include agents like peroxynitrite anion (ONOO-) and nitric oxide ($NO^{\bullet}$) radicals. In addition, there are non-free radical species such as hydrogen peroxide (HOOH), nitric oxide (NO) and hypochlorous acid [1]. These molecules plays important role in cellular injury and aging process. Various pathological conditions such as cancer, cataracts, chronic inflammation; as well as, diabetes mellitus, cardiovascular and neurodegenerative diseases believed to be associated with oxidative stress [2,3,4].

Antioxidants are compounds that neutralize free radicals which otherwise damages the body. Synthetic antioxidants such as butylated hydroxyanisole(BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone, and propyl-gallate has been widely used. The incorporation of these synthetic antioxidants in food preparations have been questioned due to potential health risks, toxicity and carcinogenicity [5,6]. Plant materials contain numerous types of antioxidants with varied activities. In recent years much attention has been devoted to natural

antioxidant and their association with health benefits and many plants exhaustively studied in the last few years for their antioxidant and radical scavenging activities [7].

Therefore, taking into consideration the vast potentiality of plants as sources for antioxidants a systematic investigation was undertaken to screen the local flora for radical scavenging activity. Seeds of *Artocarpus heterophyllus* Lamk and leaves of *Balanites aegyptiaca* L , extracted in different solvents and tested for radical scavenging activity against 2, 2-diphenyl-1-picrylhdydrazyl (DPPH). Ascorbic acid (Vit.C) was used as antioxidant reference compounds. Preliminary phytochemical investigation was also carried out.

Material and Methods

Collection of plants and authentication :

Seeds of *Artocarpus heterophyllus* and leaves of *Balanites aegyptiaca* were collected from Akola to Shegaon road, near about 14 km from Akola. The collection was done at the mid day, in the month of December 2010. Collected plant parts were shed dried at room temperature and pulverized into powdered form and stored in to pockets at room temperature. The plants were identified by its vernacular name and later identified and authenticated by Dr. N. Dongarwar of Botany department R.T.M. Nagpur University, Nagpur and a herbarium was deposited at the Botany Department. The voucher specimen numbers are--
9520 - Artocarpus heterophylus Lamk. *9521 - Balanites aegyptiaca* L.

Extraction

40 gm of each powdered plant materials were extracted with 500 ml of various solvents like water, acetone and chloroform separately by keeping for 7-15 days on a rotary shaker. An intermediate shaking was done for proper extraction. Mixtures were filtered through filtration assembly and dried under reduced pressure. The fine powder obtained for each extracts were kept for the further tests. The extracts obtained were shown in table no. 1.

Determination of Antioxidant activity (DPPH free radical scavenging activity)

Anti-oxidant activity of the extract was investigated on the basis of their scavenging potential of the 2, 2- diphenyl-1-picryl hydrazyl (DPPH) free radical. DPPH is most extensively used and easiest method to determine antioxidant activity [8.] Substance capable of donating hydrogen atom or having antioxidant activity on mixing with solution of DPPH in methanol, gives reduced form with the loss of violet color ,a pale yellow color formation is also occurs some time due to picryl group [9].

Various plant extracts were prepared with concentrations of 0.02, 0.04, 0.06, 0.08, 0.10 mg/ml in methanol. Ascorbic acid (Vit. C) at same concentrations was used as the antioxidant standard. 1 ml of the extract prepared was placed in a test tube, and 0.5 ml of 0.1 mM DPPH in methanol is added. It is further diluted with 1.9 ml of methanol. A solution of the same amount of methanol and DPPH is kept as the blank solution. The absorbance was noted at 517nm on spectrophotometer after incubation period of 20-30 minutes. The radical scavenging activity was calculated using the following formula:

% inhibition = { [Ab-Aa] / Ab} x 100
Where, Ab is the absorption of the blank sample and,
Aa is the absorption of the extract

Preliminary Phytochemical analysis.

Plants are considered as biosynthetic laboratory for a multitude of secondary metabolites like alkaloids, glycosides, polyphenols, volatile oils, tannins, etc. that exerts physiological effects. Preliminary screening of extracts for phytoconsituents were done for possibly obtainable various bioactive constituents and results are noted [10, 11].

Table no.1: The plant, its part, solvents used for extraction.

Sr.N	Plant	Part	Solvent	Abbreviations
1	*Artocarpus heterophylus* Lamk.	Seed	Acetone	AcSA
2	*Artocarpus heterophylus* Lamk.	Seed	Chloroform	ChSA
3	*Balanites aegyptiaca* L.	Leaves	Acetone	AcLB
4	*Balanites aegyptiaca* L.	Leaves	water	AqLB

Table No 2 . For Vitamin C (standard)

Sr. No.	Concentrations (mg/ ml)	Absorbance (517nm)	% inhibition
1	Blank	0.121	-----
2	0.02	0.075	38.016
3	0.04	0.062	48.760
4	0.06	0.058	52.066
5	0.08	0.057	52.892
6	0.10	0.056	53.719

Table no.3 For aqueous extract of leaves of *Balanites aegyptiaca.*

Sr. No.	Concentrations (mg/ ml)	Absorbance (517nm)	% inhibition
1	Blank	0.121	-----
2	0.02	0.089	26.446
3	0.04	0.087	28.099
4	0.06	0.084	30.578
5	0.08	0.077	36.363
6	0.10	0.070	42.148

Table no 4. For acetone extract of leaves of *Balanites aegyptiaca.*

Sr. No.	Concentrations (mg/ ml)	Absorbance (517nm)	% inhibition
1	Blank	0.121	-----
2	0.02	0.116	4.132
3	0.04	0.114	5.785
4	0.06	0.113	6.611
5	0.08	0.112	7.438
6	0.10	0.105	13.223

Table no. 5. For acetone extract of seed of *Artocarpus heterophylus* Lamk.

Sr. No.	Concentrations (mg/ ml)	Absorbance (517nm)	% inhibition
1	Blank	0.121	-----
2	0.02	0.115	3.809
3	0.04	0.113	5.714
4	0.06	0.111	7.619
5	0.08	0.107	11.428
6	0.10	0.106	12.380

Table no. 6. For chloroform extract of seed of *Artocarpus heterophylus* Lamk.

Sr. No.	Concentrations (mg/ ml)	Absorbance (517nm)	% inhibition
1	Blank	0.121	-----
2	0.02	0.115	4.958
3	0.04	0.114	5.785
4	0.06	0.113	6.611
5	0.08	0.106	12.396
6	0.010	0.103	14.876

Table No.7. Preliminary phytochemical analysis of various tested plant extracts.

Sr. No.	Plant constituent	Extracts			
		AqLB	AcLB	AcSA	ChSA
1	Taninns	+	+	+	+
2	Terpenoids	+	+	+	+
3	Sterols	_	_	+	+
4	Saponins	+	_	_	+
5	Anthraquinones	_	_	+	+
6	Flavonoides	_	_	_	_
7	Reducing sugars	_	_	_	_
8	Cardiac Glycosidase	_	_	_	_
9	Alkaloids	_	_	_	_

Results

The reduction capability of the DPPH radicals was determined by the decrease in absorbance at 517 nm. Among the various extracts used in this study for evaluation of antioxidant activity, aqueous extract of leaves of *Balinites aegyptiaca* (AqLB) and acetone extract of seed of *Artocarpus heterophylus* . (AcSA) showed highest and lowest antioxidant activity respectively. Absorbance values and percent inhibition of each extracts and vitamin C were shown in table no.2-6. Radical scavenging activity of 42.148 and 12.396 % at tested concentration 0.1 mg/ml were reported for prior and later extracts. Choroform extracts of seed of *Artocarpus heterophylus* . (ChSA) have antioxidant activity of 14.876 % followed by acetone extract of leaves of *Balinites aegyptiaca* (AcLB) with 13.223 % antioxidant activity. The results of the preliminary phytochemical investigation for obtained four extracts were shown in table no.7.

Discussion and Conclusion

Aqueous extract of leaves of *Balinites aegyptiaca* . (AqLB) have more antioxidant activity than that of acetone extract of leaves of the same plant. Also, Chloroform extract of seed of *Artocarpus heterophylus* (ChSA) is having more free radical scavenging activity than that of Acetone extract of seed of the same plant. Ascorbic acid (Vit.C), a standard used in the experiment showed antioxidant activity of 53.719 %. Concomitant increase in radical scavenging activity was observed with increase in concentration of the extracts from 0.02 to 0.1 mg/ml, including ascorbic acid (Vit.C). Radical scavenging activity of 38.016 % and 53.719 % were observed for standard, corresponding to the concentration of 0.02 and 0.1 mg/ml respectively. Antioxidant activity reported for all tested plant extracts were in the range of 12 to 14% except for Aqueous extract of leaves of *Balinites aegyptiaca* (AqLB) at highest tested concentration of 0.1 mg/ml. Aqueous extract of leaves of *Balinites aegyptiaca* (AqLB) showed almost three times more percent inhibition and can be proved as strong antioxidative agent over the other plant extracts.

Investigation for possible bioactive compounds present in the test extracts revealed some differences in the constituents. Acetone extract (AcSA)and chloroform extract for seed of *Artocarpus heterophylus* (ChSA) showed presence of sterols and anthraquinones which was not reported in Aqueous extract of leaves of *Balinites aegyptiaca* (AqLb) and Acetone extract of leaves of *Balinites aegyptiaca* (AcLB). Tannins and terpenoids were reported in all plant extracts, whereas test for alkaloids, flavonides, reducing sugars and cardiac glycosides were negative. Earlier studies attributed the antioxidant activity of plants to the presence of these secondary metabolites [12]. Recently many natural antioxidants have been isolated from different plant materials[13]. Saponins, SAP-1016 (3β-O-β-d-xylopyranosyl-(1–3)-β-d-glucopyranosyl-(1–4)-[α-l-rhamnopyranosyl-(1–2)]-β-d-glucopyranoside, already isolated from *Balanites aegyptiaca* and studied for its pharmacological activity[14]. Aqueous extract from *Balinites aegyptiaca* leaves showed presence of saponins and its absence in acetone extract of the same indicate a possible role of saponins in high radical scvavenging activity of prior extract compare to later. It may also be possible that the combined effect (synergistic or antagonistic) of saponins with other phytochemicals may responsible for observed antioxidant activity.

This study indicates, a potential for the development of antioxidative agent from these plants extracts especially, with aqueous extracts from laves of *Balinites aegyptiaca*. Although some of these constituents have already been isolated from these plants, there exists a possibility for a new compounds possessing anti-oxidative activity and further study is needed for their isolation and structural characterization.

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