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Evaluation of *In-vitro and In–vivo* Anti-oxidant Activity of Roots of *Kandelia rheedei* and Leaves of *Euphoria lopogona*

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A B S T R A C T

The literature survey shown Mangrove and Cactus Plants were also have the tremendous important in the treatment of the disease. Roots of Kandelia rheedei (Rhizophoraceae) and Leaves of Euphorbia lopogona (Euphorbiaceae) were selected for the evaluation of anti-oxidant activity. Phytochemical screening revels that the plants having the flavonoids, tannins, proteins and phenols. From the above information plants shows anti-oxidant, hepatoprotective, antidiabetic and anti hyperlipidamic activity. DPPH method, Lipid-peroxidation method , the ethyl acetate fraction of both plant extracts shown significant free radical scavenging activity, the IC_{50} value were decreased when concentrations of fractions increased this values indicates the potency of the herbs showing the anti-oxidant activity.

Keywords: Anti-oxidant activity, Kandelia rheedei, Euphorbia lopogona, DPPH method, Lipid peroxidation method.

A R T I C L E I N F O

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1. Introduction

Herbal medicines are popular as remedies for diseases and play a key role in the human health care of a vast majority of world's population. According to WHO as much as 4 billion people (80%) of the world's populations rely on the use of traditional medicine which is predominantly based on plant material. The traditional medicine refers to a broad range of ancient natural health care practices including folk / tribal practices, as well as, Ayurveda, Siddha, Amchi and Unani. These medicinal practices have originated from time immemorial and developed gradually, to a large extent, by relying or based on practical experiences, without significant references to modern scientific principles. Although herbal medicines are effective in the treatment of various ailments, very often those drugs are unscientifically exploited and /or improperly used .Therefore, those plants drugs deserved detailed studies in the light of modern sciences. India has very rich flora with nearly 16,000 species of flowering plant. It is estimated that about 7,500 herbs are used in local health traditions mostly, in rural and tribal villages of Indian. Out of those, the real medicinal value of over 4,000 plants is either little known or hitherto unknown to the mainstream population. The detailed investigation and documentation of plants and their taxonomical relatives can lead to the development of invaluable plant drugs for many dreaded diseases.

2. Materials and Methods

Plant Material:

Roots of *Kandelia rheedei* and Leaves of *Euphoria lopogona* were collected from Kothagudem forest Andhra Pradesh India between August and September; the plants were authenticated by the Professor R Venu Gopal, SR&BGNR Govt. Degree & PG College Kothagudem, Kammam Dts. A voucher specimen (SSR 2012/09/14 and SSR 2013/12/14) has been preserved in our laboratory. The plants were washed thoroughly in tap water, shade dried and powdered.

Preparation of Extracts:

The air-dried roots of Kandeliarheedei Linn. (1.5 kg) and leaves of *Euphorbia lopogona* Linn. (2.5 kg) were coarsely powdered separately. Then the coarsely powdered material of each plant was extracted with methanol by maceration for 7 days in round bottom flasks at room temperature separately. Methanolic extract of each plant was dispersed in 1 L of water separately and fractionated with toluene, ethylacetate, butan-2-one and n-butyl alcohol in succession. The solvents were removed from the fractions under reduced pressure to yield the corresponding extracts.

Maintenance of Animals:

Wister rats, weighing about 150–200 g and mice weighing 22-25 g were obtained from the Mahaveer Enterprises, Bagh Ambarpet, Hyderabad (CPCSEA registration no: 146/1999/cpcsea) and the animals were kept in the animal house of Sree College of Pharmacy, Nayakula gudem, kotha gudem Kammam A.P - 507020 at room temperature of 25 - 30°C and at 45 - 55% relative humidity for 12 hr, each of dark and light cycle. The animals were feed with rat pellets (Hindustan Lever Limited, Bangalore, India) and filtered water. Animal studies in the work have been strictly performed as per the Institutional Animal Ethical Committee (IAEC).

Plant Profile

Kandelia rheedei L

Kandelia rheedei is the Mangrove plants found in the coasts of South Asia and Southeast Asia, from western India to Borneo has been proven as anti diabetic drug. The medicinally active parts of the plant are the leaves and the roots although the exact mechanism is unknown.

Euphoria lopogona

A member of the Cactus family (Family: Euphorbiaceae) *Euphoria lopogona* is a woody plant found in tropical forests of India and Africa has been proven as ant diabetic drug. The medicinally active parts of the plant are the leaves and the roots although the exact mechanism is unknown. Besides impairing the ability to discriminate sweet taste increase enzyme activity responsible for the glucose uptake and utilization. It may stimulate pancreatic cell function, increase cell number and increase insulin release by increasing cell permeability to insulin.

Assessment of Anti-oxidant activity of Extracts

***In-vitro* DPPH Method**

For the estimation of antioxidant activity , different concentration (5, 10, 25, 50, 100 mg/ml) Of EAFKR, EAFEL and Ascorbic acid in methanol were added to 100 ml of 0.2 mm Methanolic solution of DPPH, mixed thoroughly and 20ml of the mixture was used for HPLC Analysis. Each test was performed in triplicate, which was carried out using reverse phase C_{18}Column equipped with waters UV/Visible Spectrophotometric detector. Isocratic elution conditions for HLPC analysis were methanol: water (85:15) monitored at 517NM.(Abe et al., 2000) . The antioxidant activity was measured in terms of % inhibition of DPPH peak area. The amount of drug required to produce 50% inhibition of DPPH peak area was taken as IC_{50} . The IC_{50} values computed from concentration of test extract and percent inhibition of DPPH peak area.

***In-vivo* study in steptozotocin induced diabetic rats (sub acute model) by estimation of Lipid-peroxidation product (malondialdehyde):**

The amount of lipid peroxidation product (MDA) malondialdehyde present in the serum samples drawn from the streptozotocin induced diabetic control, EAFKR and EAFEL treated diabetic rats 1st and after 28 days of the study was estimated by the Thiobarbituric acid reactive aubstances (TBARS) method (Carbineau,1991), which measures the malondialdehyde (MDA) reactive products by using High Pressure Liquid Chromatography (HPLC).

Procedure:

To 0.5 ml of 30% trichloro acetic acid (TCA) was added to precipitate the proteins and vortexed for 30sec. Clear supernatant was taken after centrifuging at 3000 rpam for 10 min. To the supernatant 50µl of 1%TBA solution was heated for 1Hr AT 98^{O} C. 20µ of the mixture, which is pink in color, was injected into HPLC. Standard raph was plotted using TEP (1,1,3,3-tetra ethoxy propane).

Statistical Analysis:

All the group were statistically evaluated using one way analysis of variance (ANOVA) followed by Newman's Keull multiple comparision test, expressed as the Mean ± SD from six rats in each group. P value of 0.05 or less was considered to be significant.

3. Results and Discussion

Antioxidant studies on EAFKR and EAFEL

***In-vitro* study by DPPH:**

The percentage inhibition of DPPH peak area by ascorbic acid, EAFKR and EAFEL are summarized in Tables. Antioxidant activity was measured in terms of percentage inhibition of DPPH peak area. The test extract, ascorbic acid, the reference compound increased percentage inhibition of DPPH peak area in a concentration dependent manner. EAFKR and EAFEL at 100 µg/ml concentration showed 62.56% and 99.42 % inhibition respectively, where as the reference compound, ascorbic acid at the same concentration exhibited 93.5% inhibition. Among the three extract, EAFKR was found to be the most effective antioxidant (IC_{50} – 67.5 µg ml) followed by EAFEL (IC_{50} – 59.74 µg ml) However, the IC_{50} value of ascorbic acid (IC_{50} – 21.07 µg ml) was found to be lower than those of the test extracts.

Table 1: Antioxidant activities of ethyl acetate fractions of KR and EL by DPPH method

Conc. (µg/ml)	% Inhibition of DPPH peak area		
	Ascorbic acid	EAFKR	EAFEL
5	18.69+1.4	8.06±0.8	7.62±0.2
10	30.81±1.8	13.25±1.2	18.07±1.4
25	51.75±2.3	31.04±2.9	34.63±2.7
50	79.8±3.6	44.53±3.5	59.18±3.6
100	93.5± 4.6	62.56±3.2	79.42+5.2

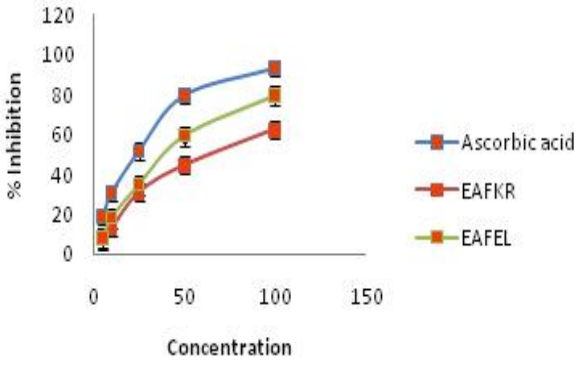

Figure 1

***In-vivo* study in streptozotocin induced diabetic rats (sub acute model) by estimation of lipid-peroxidation product (malondialdehyde)**

The results of the study are shown in Tables the level of malondialdehyde was significantly increased in diabetic control rats. Administration of EAFKR and EAFEL to diabetic rats daily at 100 mg/kg b.w. dose for 28 days significantly ($p<0.001$) decreased the level of lipid peroxidation marker, malondialdehyde after 28 days. EAFKR and EAFEL exhibited the same degree of antioxidant activity.

Table 2: Effect of EAFKR and EAFEL on MDA level

Group	Dose (Mg/kg b.w.)	Fist Day ((nM/m1)	Day 28 ((nM/m1)
control	-	2.43±0.21	2.98±0.17
EAFKR	100	2.87±0.10	1.15±0.09***
EAFEL	100	2.74±0.17	1.93±0.18***

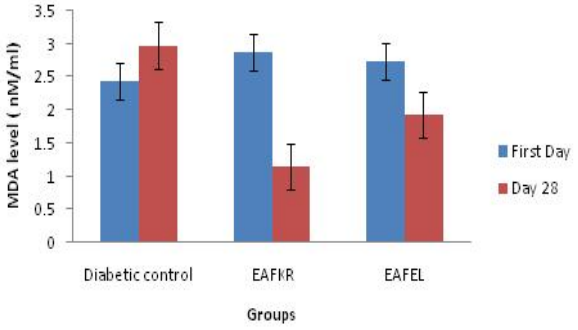

Figure 2

***P< 0.001 in comparison with Diabetic control

Discussion

a. Methanolic extract of roots of *Kandelia rheedei (*MKR*) and* leaves of *Euphoria lopogona (*MEL*)* were found to be non-toxic in albino mice up to 2 g/kg b.w. p.o.
b. MKR and MEL at a dose of 400 mg/kg b.w. showed significant oral glucose tolerance in normal rats.
c. EAFKR and EAFEL at dose, 100 mg/kg b.w. showed significant oral glucose tolerance in streptozotocin induced type 2 diabetic rats.
d. In *in-vitro* study EAFKR and EAFEL exhibited a concentration dependent free radical scavenging activity. In sub acute study in streptozotocin induced diabetic rats, EAFKR and EAFEL showed antioxidant activity by significantly decreasing the level of lipidperoxidative-marker, malondialdehyde (MDA), a protective action against cell damage required in diabetes therapy.

4. Conclusion

The studies revealed that EAFKR and EAFEL shown anti oxidant activity. Thus to conclude, EAFKR and EAFEL were endowed with anti oxidant activity. The active fractions, EAFKR and EAFEL could be beneficial in the management of Free radicals, Hepatitis, type-II diabetes. Therefore, further comprehensive scientific investigations are required on these extracts.

5. References

1. Kirtikar KR, Basu BD. Indian Medicinal Plants, vol-II, 2nd Ed. DeraDun Publisher Ltd: New Delhi, **1987**, pp. 1549.

2. Kokate CK. Practical pharmacognosy. 1st edition, Vallab prakashan. New Delhi, **1986**, pp.111-113.
3. Bhakuni DS, Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN. Screening of Indian plants for biological activity part II. Indian Journal of Experimental biology. **196**9, 7(4) 250-262.
4. Bhatt, In: Healing Plants of Peninsular India. J.A.Parrotta.CAB International, **2001**, 855.
5. Blios. Antioxidant determinations by the use of a stable free radical. Nature. **1958**, 26: 1199.
6. Bruhn J, Holmshedt B. Ethnopharmacology: Objectives, principles and perspectives.In: Beal JL, Reinhard E, editors. Natural Products as Medicine. Hippokrates Verlag 405-430; (1981).
7. Chandan KS. Oxygen toxicity and antioxidants. Ind J Physiol Pharmacol Toxicol. **1995**, 9: 419
8. Chopra. In: Healing Plants of Peninsular India. J. A. Parrotta.CAB International. **2001**; 255-56.
9. Evans PH. Free radicals in brain metabolism and pathology. In: Cheeseman KH, Slater TF editors. Free radicals in medicine. Churchill Livingstone, London, **1992**; pp. 577-587.
10. Fita I. Rossmann MG. The active centre of catalase. J Mol Biol., **1985**, 185: 21-37.
11. Gunta SS. Prospects and Perspectives of natural products in medicine. *Indian J Pharmacol.* **1994**, 26: 1-12.
12. Halliwell B, Gutteridge JMC. The antioxidant of human extracellular fluids. *Arch Biochem Biophys.* **1992**, 280: 1-6.