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Evaluation of Antioxidant, Anti-inflammatory and Analgesic Potential of the *Luffa acutangula* Roxb. Var. amara

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ABSTRACT

Natural products are always helpful in the maintenance of life and good health. The aim of the present study was to evaluate *Luffa acutangula* Roxb. var. amara ethanolic seed extract for antioxidant activity by 1,1-Diphenyl-2-picryl hydrazyl and Hydrogen peroxide method, anti-inflammatory by Carrageenan induced rat paw edema method and analgesic activity by tail flick and tail immersion methods. The extract showed potent antioxidant activity i.e., 75.33 ± 0.592 and $76.50 \pm 0.281\%$ at $200 \mu\text{g mL}^{-1}$ by 1,1-Diphenyl-2-picryl hydrazyl and Hydrogen peroxide method as compared to ascorbic acid. The extract showed significant antiinflammation i.e., 60.8 at 300 mg mL^{-1} as compared with standard (diclofenac sodium). Further the extract showed significant analgesic activity and the reaction time noted was 6.25 ± 0.52 and 5.80 ± 0.52 at a dose of 400 mg mL^{-1} as compared to standard (pentazocin). The above results concludes that ethanolic seed extract of *Luffa acutangula* Roxb. var. amara can be used as natural antioxidant, inflammation and in the treatment of pain.

Key words: *Luffa acutangula* Roxb. var. amara, antioxidant, anti-inflammatory, analgesic, hydrogen peroxide

INTRODUCTION

Nature is an immense source of pharmacologically active molecules which has been used for the treatment of the incurable diseases without any adverse effects (Kokate *et al.*, 2000; Ravi *et al.*, 2009; Trease and Evans, 1983). Ayurvedic plants are more potent and known to have human medicinal properties (Najiah *et al.*, 2011; Nithya and Baskar, 2011). Indian system of medicines has been recommended for different kind of biological activity for their health care needs (Junaid *et al.*, 2011; Al-Mustafa and Al-Thunibat, 2008). The products obtained from the plant source are clinically used for health benefits without any chemical modification (Nenaah and Ahmed, 2011). According to World Health Organization, more than 21,000 plants have been used as medicinal agent around the world (Joseph and Raj, 2011). There are lots of plants to be explored for their pharmacological activity (Archana *et al.*, 2011). An 80% of the world population employs herbal medicines to deals with their daily medicinal issue and they have negligible adverse effect as compare to modern medicine (Karim *et al.*, 2011; Premanath *et al.*, 2011; Kapoor and Saraf, 2011). They have great value to phytochemists because of their medicinal properties (Oladosu *et al.*, 2011). Phytoconstituents are the immense source of natural antioxidants (Khasawneh *et al.*, 2011). Natural antioxidants counteract and detoxify these Reactive Oxygen Species (ROS) in order to survive and appear to contribute chemo preventive or chemo protective activity by maintaining

homeostatic balance between Reactive oxygen species and endogenous antioxidants (Chiang *et al.*, 2004). Free radicals are also responsible for induction of short time analgesia and moreover free radical plays an important role in the pathogenesis of inflammation (Rokyta *et al.*, 2003; Winrow *et al.*, 1993). Inflammation is a normal defense response of cells in which various complex biological response of vascular tissue occurs to harmful stimuli (Alam *et al.*, 2011; Susithra *et al.*, 2010).

Many studies showed that flavonoids present in seed extracts, possesses significant antioxidant activity and appears to be associated with a reduced risk for certain chronic diseases, such as cancer, inflammation and ulcer (Zayachkivska *et al.*, 2005). Hence, the present study has been conducted to explore *Luffa acutangula* Roxb. var. amara seed as natural antioxidant, anti-inflammatory and analgesic activity.

MATERIALS AND METHODS

Chemicals: Hydrogen peroxide, ascorbic acid and 1,1-diphenyl-2-picrylhydrazyl were obtained from S.D. Fine chemicals Ltd., Diclofenac sodium, Gum acacia and Pentazocin was produced from Spectrochem Pvt. Ltd., Mumbai, India. All other chemicals were of analytical grade and obtained from local sources.

Plant material: The seeds of *Luffa acutangula* Roxb. var. amara were collected from local grain market in the month of Sep 2010 from Sector 26 Chandigarh. It was authenticated and the specimen No. 0392/Herb was deposited in Botanical and Environmental Science Department, Guru Nanak Dev University, Amritsar (PB). The seeds were coarsely powdered and passed through sieve No. 40. It was used for further analysis.

Preparation of extract: Dried (100 g) coarse powdered seed were extracted for 24 h with aqueous ethanol (70% v/v) by simple maceration process. The successive aqueous ethanolic extract was filtered and dried under reduced pressure to get a solid mass free from the solvent. The solvent was evaporated to dryness under reduce pressure by rotary evaporated at 40°C. The concentrated extract was collected and stored in the refrigerator at 4°C.

Animals used: Adult albino rats (30) weighing between 150-200 g and Adult Swiss albino mice (48) weighing between 25-30 g of either sex were used for the studies. The animals were maintained under normal laboratory condition and kept in standard polypropylene cages at room temperature of 30±2°C and 60 to 65% relative humidity and provided standard diet and water. The animals were acclimatized to laboratory condition for one week prior to experimentation. The experimental protocols were approved by institutional Animal Ethical Committee (Reference No.-874/ac/05/, CPCSEA).

Phytochemical screening: The phytochemical screening of extract was carried out as per the standard procedures (Mohan and Sanjay, 2010; Gill *et al.*, 2010).

Antioxidant activity

Quantitative scavenging activity on 1,1-Diphenyl-2, picrylhydrazyl radical: The antioxidant activity was measured by 1,1-Diphenyl-2, picrylhydrazyl radical method (Hatano *et al.*, 1988):

$$\text{Radical scavenging activity (\%)} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Hydrogen peroxide scavenging activity: The hydrogen peroxide scavenging activity of extract was determined by using the method of Czochra and Widensk (2002):

$$\text{Percentage H}_2\text{O}_2 \text{ scavenging activity} = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100$$

Where:

Ac = Absorbance control

As = Absorbance sample

Acute inflammation study: Anti-inflammatory activity was evaluated using Carrageenan induced rat paw edema method. Six groups were employed in the present study each group consists of six rats:

- **Group I:** Vehicles control group (1% CMC, p.o.)
- **Group II:** Disease control group (0.1 mL of Carrageenan, 1% injected in the plantar surface of the right hind paw)
- **Group III:** Reference standard (diclofenac sodium, 10 mg kg⁻¹)
- **Group IV-VI:** Ethanolic seed extract of *Luffa acutangula* var. amara at different dose level (100, 200 300 mg kg⁻¹, p.o.)

All the groups were employed in paw edema test by using plethysmometer and paw volume was measured at 0, 1, 2 and 3 h intervals (Ashutosh *et al.*, 2009; Winter *et al.*, 1962).

Analgesic activity by tail flick method: Swiss albino mice were screened for sensitivity test by placing the tip of the tail on the radiant heat source before the conduct of study. Any animals that held to withdraw its tail in 5 sec were rejected from the study (Agrahari *et al.*, 2010).

Analgesic activity by tail immersion Test: Prior to analgesic experiments, the animals were screened for the sensitivity test by immersing the tail of the mice gently in hot water maintained at 52.5±0.5°C. The animal immersing the tail from hot water within 5 sec was selected for the study according to the method of Geetha *et al.* (2004).

Statistical analysis: All the biochemical results were expressed as mean± standard error of means (SEM). Data were analyzed by Turkey's multiple test range using Sigma Stat Version-3.5 software. A probability value of p<0.05 was considered to be statistically significant.

RESULTS

The phytochemical screening of seed extract of *Luffa acutangula* Roxb. var. amara indicates the presence of triterpenoids, carbohydrates, coumarin glycoside and Phenolic compound phytoconstituents (Table 1).

Antioxidant potential of ethanolic seed extract was evaluated by 1, 1-diphenyl-2-picrylhydrazyl and Hydrogen peroxide method. Antioxidant activity of ethanolic seed extracts

of *Luffa acutangula* Roxb. var. amara. was 75.33 ± 0.592 by 1,1-diphenyl-2-picrylhydrazyl model as shown in Table 2 and 76.50 ± 0.281 by hydrogen peroxide method as shown in Table 3 at $200 \mu\text{g mL}^{-1}$ as compared to ascorbic acid. The reduction capability of 1,1-diphenyl-2-picrylhydrazyl and Hydrogen peroxide free radical was determined by the decrease in its absorbance at 517 and 230 nm, respectively.

The anti-inflammatory activity was estimated by Carrageenan induced rat paw edema method the extract showed significant percentage inhibition i.e., 60.8 at 300 mg mL^{-1} as compared with standard (diclofenac sodium) which is mentioned in Table 4.

Further it was evaluated for analgesic activity and the extract showed significant analgesic activity the reaction time noted was 6.25 ± 0.52 by tail flick method and 5.80 ± 0.59 by tail immersion methods at a dose of 400 mg mL^{-1} as compared to standard (pentazocin) which is mentioned in Table 5 and 6, respectively.

Table 1: Phytochemical screening of *Luffa acutangula* Roxb. var. amara

Chemical constituents	Ethnolic extract of <i>Luffa acutangula</i>
Alkaloids	-
Steroids	+
Tannins	+
Flavonoids	++
Glycoside	-
Starch	++

++: High content, +: Moderate, -: Negative

Table 2: Free radical scavenging activity of *Luffa acutangula* Roxb. var. amara, by using 1,1-Diphenyl -2 picrylhydrazyl method

Concentration of extract ($\mu\text{g mL}^{-1}$)	DPPH	Ascorbic acid
50	48.80 ± 0.351	50.23 ± 0.651
100	60.22 ± 0.532	74.50 ± 0.792
200	75.33 ± 0.592	85.40 ± 0.493

Values are mean of triplicate experiments and represent as Mean \pm SEM

Table 3: Free radical scavenging activity of *Luffa acutangula* Roxb. var. amara by using hydrogen peroxide

Concentration of extract ($\mu\text{g mL}^{-1}$)	Hydrogen peroxide	Ascorbic acid
50	71.23 ± 0.921	74.50 ± 0.690
100	65.80 ± 0.679	69.00 ± 0.720
200	76.50 ± 0.281	78.00 ± 0.678

Values are mean of triplicate experiments and represent as Mean \pm SEM

Table 4: Anti-inflammatory activity of ethanolic seed extracts of *Luffa acutangula* Roxb. var. amara (EELA)

Group (mg kg^{-1})	Mean paw volume			% inhibition
	1 h	2 h	3 h	
Disease control	0.52 ± 0.002	0.63 ± 0.0024	0.72 ± 0.0957	-
Diclofenac sodium	0.40 ± 0.0073^a	0.43 ± 0.0041^a	0.54 ± 0.056^a	25
EELA(100)	0.46 ± 0.0085^b	0.55 ± 0.0062	0.65 ± 0.0045	10
EELA(200)	0.41 ± 0.029^b	0.49 ± 0.019^{ab}	0.62 ± 0.056^a	13.88
EELA(300)	0.38 ± 0.25^a	0.46 ± 0.016^{ab}	0.27 ± 0.010^a	60.8

Values are Mean \pm SEM of 6 animals in each group, ^ap<0.05 vs. control, ^bp<0.05 vs. diclofenac sodium

Table 5: Analgesic activity of *Luffa acutangula* Roxb. var. amara seeds by Tail flick method

Treatment	Dose	Basal reaction time (sec)	Reaction time (min)			
			15	30	45	60
Control	2 mL kg ⁻¹	2.50±0.38	2.60±0.40	2.70±0.30	2.78±0.40	4.38±0.30
pentazocin	5 mg mL ⁻¹	2.44±0.18	4.12±0.12 ^a	5.62±0.32 ^a	7.10±0.22 ^a	5.50±0.40 ^a
EELA	200 mg kg ⁻¹	2.35±0.16	3.59±0.18 ^b	4.62±0.22 ^b	6.12±0.24 ^b	7.29±0.40 ^b
EELA	400 mg kg ⁻¹	2.68±0.35	3.83±0.26 ^b	4.92±0.42 ^b	6.62±0.40 ^b	6.25±0.52 ^b

Result expressed as Mean±SEM from six observations, indicates ^ap<0.01 compared with control group, ^bp<0.05 compared with pentazocin treated group

Table 6: Analgesic activity of *Luffa acutangula* Roxb. var. amara seeds by Tail immersion method

Treatment	Dose	Basal time reaction (sec)	Reaction time (min)			
			15	30	45	60
Control	2 mL kg ⁻¹	2.50±0.38	2.60±0.40	2.70±0.30	2.78±0.40	4.30±0.30
pentazocin	5 mg mL ⁻¹	2.44±0.18	4.12±0.12 ^a	5.62±0.32 ^a	7.10±0.22 ^a	5.10±0.40 ^a
EELA	200 mg kg ⁻¹	2.35±0.16	3.59±0.18 ^b	4.62±0.22 ^b	6.12±0.24 ^b	7.20±0.40 ^b
EELA	400 mg kg ⁻¹	2.68±0.35	3.83±0.26 ^b	4.92±0.42 ^b	6.62±0.40 ^b	5.80±0.59 ^b

Result expressed as Mean±SEM from six observations, indicates ^ap<0.01 compared with control group, ^bp<0.05 compared with pentazocin treated group

DISCUSSION

Luffa acutangula Roxb. var. amara seed extract showed potent antioxidant activity. The seed oil from the of *Luffa cylindrica* had shown significant antioxidant, anti-inflammatory and analgesic activity (Yoganandam *et al.*, 2010). It may be because of their ability to neutralize free radicals, reactive oxygen species or oxidant.

Further the seed extract showed significant anti-inflammatory activity. The seed of *Cucumis melo* a cucurbits species, had shown antioxidant anti-inflammatory and analgesic activity (Gill *et al.*, 2011). The development of Carrageenan induced edema is believed to be biphasic (Vinegar *et al.*, 1976). Carrageenan model is mediated mainly by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorpho-nuclear cells and prostaglandins produced by tissue macrophages (Antonio and Brito, 1998).

Furthermore, it was evaluated for analgesic activity and the extract showed significant reduction in pain. Methanolic and aqueous fruit extract of *Lagenaria siceraria*, a cucurbits species, had shown significant analgesic activity (Shah and Seth, 2010). The analgesic activity may be due to its free radical scavenging activity as their free radicals are involved during pain stimulation (Kim *et al.*, 2004). Analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain (Pal *et al.*, 1999; Elisabetsky *et al.*, 1995). In tail flick method and tail immersion method pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems (Rolland *et al.*, 1991; Headley and Shaughnessy, 1985). Moreover, phytochemical screening revealed that presence of flavonoids and Phenolic acid in the seed extract, these constituents are responsible for the antioxidant, anti-inflammatory and analgesic activities and may contribute to modern world nutraceuticals (Galati *et al.*, 1994; Hanasaki *et al.*, 1994).

CONCLUSION

From the present study, it is quite apparent that ethanolic seed extract of *Luffa acutangula* Roxb. var. amara possesses significant antioxidant, anti-inflammatory and analgesic effect against different stimuli. This is evidenced by significant increase in the reaction time by stimuli in different experimental models and having significant antioxidant, anti-inflammatory and analgesic activity. Apart from the huge number of research studies in the field of antioxidant, anti-inflammatory and analgesic discovery, the field still needs more attention from scientists around the world.

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