



Journal of Medical Sciences

ISSN 1682-4474

science
alert

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JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

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Evaluation of Ethanolic Seed Extract of *Lagenaria siceraria* for Their Therapeutic Potential

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During the past few decades, the drugs from natural products have gained importance in the field of medicine. Many plants and their products exhibit marked pharmacological activities. *Lagenaria siceraria* fruit is one of the important natural plant which is used in the treatment of various disorders. The present study was designed to investigate the antioxidant, anti-inflammatory and analgesic potential of ethanolic extract of *Lagenaria siceraria* seeds. The *Lagenaria siceraria* seeds were evaluated for their antioxidant potential by 1,1-diphenyl-2-picrylhydrazyl method. The extract showed significant antioxidant potential in a dose dependent manner as compared to ascorbic acid. The extract showed maximum scavenging activity i.e., 75.19% at 200 µg by 1,1-diphenyl-2-picrylhydrazyl free radical scavenging method. Thus, it was further studied for anti-inflammatory activity by Carrageenan induced rat paw edema and analgesic activity by Tail immersion method and Hot plate method at different concentrations i.e., 100, 200 and 300 mg kg⁻¹. The results showed that ethanolic extract of *Lagenaria siceraria* seeds possess good therapeutic potential and may be useful as a natural antioxidant and it can be used in the treatment of inflammation and pain.

Key words: *Lagenaria siceraria*, antioxidant activity, natural antioxidant, therapeutic potential, inflammation

INTRODUCTION

Plants have been important source of medicine for thousands of years. Natural plants are rich source of potent medicinal compounds like morphine, cocaine, silymarin and digitalis. Many pharmacologically active compounds which showed antioxidant activity have been obtained from various plant parts and it is not possible to synthesize these compounds in laboratories. There is a large amount of evidence that phenylpropanoids and their glycosylated forms are powerful anti oxidants. They act either by direct scavenging of reactive oxygen and nitrogen species or by acting as chain-breaking peroxy radical scavengers (Korkina, 2007). Among the natural compounds, phenolic substances such as phenolic acids are known for their high anti-oxidative and anti-bacterial activity. Phenolic compounds possess marked pharmacological activities like anti-oxidant, free-radical scavenging, analgesic, anti-inflammatory and anti-ulcer activity (Kaur and Arora, 2009).

Oxidation reactions inside the biological systems can produce free radicals. Free radicals are the group of atoms having one unpaired electron and include hydroxyl, super oxide, nitric oxide and peroxide radicals. In our body many diseases are produced due to over production of free radicals (Sharma, 1995). These can cause damage to the cell or even cell death and can cause the diseases such as arthritis, cancer and AIDS (Dutra *et al.*, 2008). To protect body cells from harmful effects of free radicals certain defensive substances are required which can neutralize the free radicals (Jacob, 1995). Antioxidants are agents which scavenge these free radicals and prevent the damage caused by them (Lobo *et al.*, 2008). These are used in the treatment of various diseases like cancer, congestive heart failure, Parkinson's disease. These, themselves get oxidized and neutralize free radicals and they inhibits the oxidation of the substrate (Valko *et al.*, 2007). Antioxidants are of great importance these days and several plants of Cucurbitaceae family are known for their therapeutic potential.

Cucurbitaceae family is commonly known as family of gourds, melons or pumpkins composed of more than 110 genera and 650-850 species. Cucurbits are a major source of secondary metabolites. Therefore they are the largest and the most diverse plant families (Gill and Bali, 2011). In Cucurbitaceae family, several plants such as *Cucumis anguria*, *Momordica dioica* and *Zehneria scabra* are known for their medicinal benefits because they show antimicrobial activities (Kumar and Kamarj, 2010). The leaves of plant *Coccinia grandis* are used to control hyperglycemia and to treat various diseases such as biliary disorders, anorexia, cough, diabetic wounds and hepatic disorders (Bhattacharya and

Samanta, 2010; Yadav *et al.*, 2010). An earlier study showed that fruits of *Momordica charantia* show larvicidal activity against three mosquito species *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* (Singh *et al.*, 2006).

Lagenaria siceraria is another medicinally important plant of Cucurbitaceae family which is found in India, Moluccas and Ethiopia. The cultivated form of *Lagenaria siceraria* is considered to be of African and Asian origin. Traditionally, it is used as medicine in India, China, Brazil, Hawaiian island etc. It is commonly known as lauki and bottle gourd. Fruits of this plant are traditionally used for their cardioprotective, cardiostimulant, diuretic and nutritive properties. These are also used in treatment of pain, ulcer, fever, pectoral cough, asthma and jaundice (Shah and Seth, 2010a). A decoction of *Lagenaria siceraria* is employed in the treatment of anasarca, ascites and beriberi (Deshpande *et al.*, 2008). The extract of flowering plant of *Lagenaria siceraria* shows the presence of flavones-C glycosides. It contains more proportions of soluble dietary fibres which helps in lowering serum cholesterol levels (Milind and Kaur, 2011). The seed oils are good source of lipids and proteins and are used as protein supplement in human nutrition (Chinyere *et al.*, 2009). The seeds can be used for their therapeutic potential. The present study was carried out to evaluate antioxidant, anti inflammatory and analgesic potential of *Lagenaria siceraria* seeds.

MATERIALS AND METHODS

Plant material: *Lagenaria siceraria* seeds were purchased from Local Grain Market Kharar, Mohali in August 2011. The seeds were authenticated under the voucher specimen No: 1213 which has been deposited in the Botanical and Environmental Science Department, Guru Nanak Dev. University, Amritsar, Punjab. The seeds were cleaned, washed, dried at room temperature for two days and coarsely powdered. The sample was kept in light-protected conditions.

Drugs and chemicals: Ascorbic acid and carrageenan were obtained from Central Drug House Pvt. Ltd., Mumbai, India. Hydrogen peroxide was obtained from E-Merck Ltd., Mumbai. 1,1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Chemical Co., USA. All other chemical reagents used were of analytical grade which were procured from different companies (Loba Chem, Mumbai and Merck Limited, Mumbai).

Animals: The Wistar albino rats (200-250 g) and Swiss albino mice of either sex were obtained from Punjab Agricultural University (PAU) Ludhiana. They were

acclimatised in standard laboratory conditions like diet, environmental temperature and humidity. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) and care of the animals was carried out as per the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Extraction: The powdered seeds were extracted for 72 h with ethanol at room temperature and stirred occasionally. Initially, the solvent was filtered off using muslin cloth and then with Whatman filter paper and the filtrate obtained was concentrated under reduced pressure on a rotary evaporator at 40°C. The concentrated filtrate was defatted successively with hexane. The aqueous layer was separated and concentrated on the water bath. The extract was stored at 4°C for further use for various investigations.

Phytochemical screening: The crude extract of *Lagenaria siceraria* seeds was analyzed for the presence of various phytochemical constituents like alkaloids, carbohydrates, proteins, tannins, saponins, flavonoids, steroids, terpenoids, coumarin glycosides and phenolic acids using standard procedures of analysis (Harborne, 1973).

Free radical scavenging activity

Quantitative scavenging activity on, 1,1-diphenyl-2-picrylhydrazyl radical: The quantitative free radical scavenging activity of ethanolic extract of *Lagenaria siceraria* was determined by using 1,1-diphenyl-2-picrylhydrazyl method according to Gill *et al.* (2011).

Anti-inflammatory activity

Carrageenan-induced rat paw edema: The carrageenan-induced rat paw edema experiment was carried out according to Jain *et al.* (2011). Total five groups of rats were used and different doses were given to them as follows:

- **Group I: Control:** Carboxy methyl cellulose (1% CMC, p.o.)+carrageenan
- **Group II: Standard:** Diclofenac sodium (12.5 mg kg⁻¹, p.o.)+carrageenan
- **Group III: EELS 100:** Ethanolic extract (100 mg kg⁻¹, p.o.)+carrageenan
- **Group IV: EELS 200:** Ethanolic extract (200 mg kg⁻¹, p.o.)+carrageenan
- **Group V: EELS 300:** Ethanolic extract (300 mg kg⁻¹, p.o.)+carrageenan

where, EELS is ethanolic extract of *Lagenaria siceraria*.

Analgesic activity: In both models, groups of six mice in each group of either sex were used with different doses as follows:

- **Group I: Control group:** Carboxy methyl cellulose suspension (1% CMC, p.o.)
- **Group II: Standard group:** Diclofenac sodium at a dose of 10 mg kg⁻¹, p.o.
- **Group III: EELS 100:** Ethanolic extract at a dose of 100 mg kg⁻¹, p.o.
- **Group IV: EELS 200:** Ethanolic extract at a dose of 200 mg kg⁻¹, p.o.
- **Group V: EELS 300:** Ethanolic extract at a dose of 300 mg kg⁻¹, p.o.

Where, EELS is Ethanolic extract of *Lagenaria siceraria*.

Tail immersion method: The analgesic activity was determined by tail immersion method according to Ahmad *et al.* (1992).

Hot plate method: Analgesic activity of the extract was determined by using hot plate method according to the method of Shukla *et al.* (2010).

Statistical analysis: All the results were expressed as mean±standard error of means (SEM). The data was statistically analyzed by one way analysis of variance (ANOVA) followed by Tukey’s multiple range tests by using SigmaStat version 2.0 software. The p<0.05 was considered to be statistically significant.

RESULTS

Preliminary phytochemical screening for ethanolic seed extract of *Lagenaria siceraria* showed the maximum presence of chemical constituent’s flavonoids, amino acids, triterpenoids, carbohydrates, sterols and alkaloids (Table 1). Qualitatively, discoloration of DPPH indicates scavenging potential of the compound tested i.e., yellow color over purple. Quantitatively, antioxidant potential of EELS was evaluated by DPPH and H₂O₂ radical

Table 1: Phytochemical screening

Chemical constituents	Ethanolic extract of <i>Lagenaria siceraria</i> seeds
Flavonoids	+
Tannins	-
Sterols	+
Alkaloids	++
Proteins	-
Anthraquinone glycosides	-
Triterpenoids	++
Carbohydrates	+
Coumarin	-

+: Present, -: Absence, ++: Maximum presence of chemical constituents

Table 2: Percentage scavenging by DPPH method

Conc. of extract ($\mu\text{g mL}^{-1}$)	Age scavenging (%)	
	Ethanol extract	Ascorbic acid
100	62.78 \pm 0.37	65.4 \pm 0.47
150	62.99 \pm 0.52	71.7 \pm 0.42
200	75.19 \pm 0.49	85.8 \pm 0.26

Values are Mean \pm SE of triplicate experiments, DPPH: 1,1-diphenyl-2-picrylhydrazyl

Table 3: Effect of EELS on carrageenan induced paw edema in rats

Groups	Dose (mg kg^{-1})	Paw volume (mL)			Inhibition of edema (%)
		60 min	120 min	180 min	
Control	1% CMC	0.47 \pm 0.02	0.55 \pm 0.	0.69 \pm 0.008	--
Diclofenac 10 sodium	10	0.42 \pm 0.008 ^a	0.36 \pm 0.01 ^a	0.23 \pm 0.005 ^a	66.60
EELS	100	0.45 \pm 0.004 ^{ab}	0.51 \pm 0.04 ^{ab}	0.59 \pm 0.05 ^{ab}	14.49
EELS	200	0.44 \pm 0.01 ^{ab}	0.42 \pm 0.009 ^{ab}	0.39 \pm 0.02 ^{ab}	43.47
EELS	300	0.41 \pm 0.01 ^{ab}	0.37 \pm 0.1 ^{ab}	0.30 \pm 0.005 ^{ab}	56.52

CMC: Carboxy methyl cellulose, EELS: Ethanolic extract of *Lagenaria siceraria*. Values are Mean \pm SEM of 6 animals in each group, ^a p <0.05 compared with disease control group, ^b p <0.05 compared with diclofenac sodium treated group

scavenging activity. The maximum scavenging effect of ethanolic extract of *Lagenaria siceraria* extract on the DPPH radical was 75.19% at a concentration of 200 $\mu\text{g mL}^{-1}$ and was comparable to the scavenging effects of ascorbic acid (Table 2). Carrageenan administration was shown to significantly rise in the paw volume as compared to normal control group. The ethanolic seed extract (100, 200 and 300 mg kg^{-1}) exhibited statistically significant reduction of percentage inhibition of paw volume at dose dependent manner as compared to control group. At a maximum dose of 300 mg kg^{-1} ethanolic extract of *Lagenaria siceraria* showed maximum percentage inhibition of about 56.52% after 3 h. The other doses of 100 and 200 mg kg^{-1} of extract showed 14.49 and 43.46% inhibition of edema (Table 3). All the values were significant (p <0.05) in comparison with control and standard. The ethanolic seed extract were exhibited marked central analgesic effect as evidenced by significant increase in reaction time when compared to the normal control group. The ethanolic extract of *Lagenaria siceraria* extract (300 mg kg^{-1}) showed significant central analgesic activity by tail immersion method in which the reaction time of 14.43 \pm 0.08 sec was noticed after 3 h (Table 4). At dose of 100 mg kg^{-1} and 200 mg kg^{-1} the extract showed the reaction time of 7.13 \pm 0.01 and 7.13 \pm 0.01 sec, respectively after 3 h. Using hot plate method the extract showed better analgesic activity at a dose of 300 mg kg^{-1} with the reaction time of 12.20 \pm 0.01 sec after 3 h (Table 5). At dose of 100 and 200 mg kg^{-1} the reaction time of 5.14 \pm 0.09 and

Table 4: Analgesic effect of EELS by tail immersion test

Groups	Dose (mg kg^{-1})	Tail withdrawal latency (time sec)		
		60 min	120 min	180 min
Control	1% CMC	3.20 \pm 0.05	3.44 \pm 0.02	3.55 \pm 0.02
Diclofenac 10 sodium	10	12.23 \pm 0.08 ^a	16.43 \pm 0.02 ^a	17.10 \pm 0.05 ^a
EELS	100	5.10 \pm 0.05 ^{ab}	5.50 \pm 0.02 ^{ab}	5.14 \pm 0.09 ^{ab}
EELS	200	7.46 \pm 0.08 ^{ab}	10.83 \pm 0.02 ^{ab}	11.30 \pm 0.15 ^{ab}
EELS	300	10.43 \pm 0.14 ^{ab}	13.43 \pm 0.08 ^{ab}	14.43 \pm 0.08 ^{ab}

CMC: Carboxy methyl cellulose, EELS: Ethanolic extract of *Lagenaria siceraria*. Values are Mean \pm SEM of 6 animals in each group, ^a p <0.05 compared with disease control group, ^b p <0.05 compared with diclofenac sodium treated group

Table 5: Analgesic effect of EELS by hot plate method

Groups	Dose (mg kg^{-1})	Reaction time (sec)		
		60 min	120 min	180 min
Control	1% CMC	3.82 \pm 0.01	4.07 \pm 0.008	4.13 \pm 0.01
Diclofenac 10 sodium	10	8.32. \pm 0.04 ^a	12.13 \pm 0.07 ^a	13.84 \pm 0.06 ^a
EELS	100	7.07 \pm 0.03 ^{ab}	7.07 \pm 0.003 ^{ab}	7.13 \pm 0.01 ^{ab}
EELS	200	7.81 \pm 0.10 ^{ab}	9.48. \pm 0.02 ^{ab}	10.33 \pm 0.05 ^{ab}
EELS	300	10.38 \pm 0.01 ^{ab}	11.41 \pm .01 ^{ab}	12.20 \pm 0.01 ^{ab}

CMC; Carboxy methyl cellulose, EELS: Ethanolic extract of *Lagenaria siceraria*. Values are Mean \pm SEM of 6 animals in each group, ^a p <0.05 compared with disease control group, ^b p <0.05 compared with diclofenac treated group

11.30 \pm 0.15 sec, respectively was observed after 3 h. All the values were significant (p <0.05) in comparison with control and standard.

DISCUSSION

The fruit of *Lagenaria siceraria* plant has been already described as a prophylactic agent for the treatment of various cardiac disorders and used as antioxidant, antihyperlipidemic, antihyperglycemic, cardiogenic and as hepatoprotective (Deshpande *et al.*, 2008). The leaves of *Lagenaria siceraria* are also evaluated as emetic, anthelmintic and as antimicrobial agent (Badmanaban and Patel, 2010). The pulp of the fruit is considered as cooling agent, diuretic and antibilious (Shah and Seth, 2010b). The fruits, leaves, oil and seeds are edible and are used as folk medicines in the treatment of jaundice, diabetes, ulcer, piles, colitis, insanity, hypertension, congestive cardiac failure and skin diseases. The flowers are an antidote to poison. The stem bark and rind of the fruit are diuretic. The seed is vermifuge. Extracts of the plant have shown antibiotic activity. Leaf juice is widely used for baldness (Rahman, 2003). Various extracts of fruit of *Lagenaria siceraria* were found to have anti-inflammatory, analgesic, hepatoprotective, antihyperlipidemic, diuretic and antibacterial activities

(Shah *et al.*, 2010a). In foot and mouth diseases, green fruit slices are rubbed inside mouth (Mohale *et al.*, 2008). The fruits of this plant species are used for medicinal and culinary purposes. Medicinally, fruits are also used in the treatment of diabetes. Other uses include the treatment of cough, asthma, jaundice, kidney stone, colds and measles. Furthermore, the fruits have laxative and diuretic properties (Shah *et al.*, 2010b). Leaves of *Lagenaria siceraria* have been evaluated for analgesic and Central Nervous System (CNS) depressant activity (Pawar *et al.*, 2010). Aerial parts of this plant is quite safe and can be used in the treatment of the chronic diseases like diabetes without any toxicity (Saha *et al.*, 2011). Extracts of the plant *Lagenaria siceraria* were also found to possess significant hepatoprotective activity (Lakshmi *et al.*, 2011). Since fruit, stem bark, rind, aerial parts and leaves of the plant *Lagenaria siceraria* showed various pharmacological properties, so in the present study, ethanolic seed extract of *Lagenaria siceraria* was evaluated for its antioxidant activity followed by *in vivo* analgesic and anti-inflammatory activity. The phytochemical screening indicates the presence of flavonoids and phenolic compounds in ethanolic seed extract of *Lagenaria siceraria*. The presence of flavonoids is the basis for the confirmation of *Lagenaria siceraria* seeds as antioxidant agents. Antioxidant properties are revealed by *in-vitro* DPPH and H₂O₂ method. When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of violet colour. The decolourisation is due to the acceptance of electrons from antioxidant compound (Kalpna *et al.*, 2011). Hydrogen Peroxide is an oxidizer and under certain catalytic conditions it can degrade into water and oxygen by accepting electrons (Koop, 2006). Triterpenoids isolated from the various species of Cucurbitaceae family are responsible for anti-inflammatory activity (Saleem, 2009). Carrageenan induced rat paw edema method was performed to determine the anti-inflammatory activity of the extract. Carrageenan is a strong chemical use for the release of inflammatory and proinflammatory mediators like prostaglandins, leukotrienes, histamine, bradykinin, TNF- α , etc. (Amdekar *et al.*, 2012). In this model of inflammation ethanolic extract of *Lagenaria siceraria* had very consistent anti-inflammatory activity and thus showed significant decrease in the paw thickness of rat. *Lagenaria siceraria* inhibited the leukocyte influx and raised LTB₄ levels and decreases edema. It also cause significant free radical scavenging activity. Tail immersion and Hot plate methods were carried out to evaluate the analgesic potential of seed extract which show dose dependent

results. Free radicals are involved during pain stimulation and antioxidants show reduction in such pain. Thus ethanolic extract of seeds possess analgesic property by inhibiting the free radical formation.

There are many limitations associated with the above mentioned methods with which various pharmacological activities have been estimated. Owing to the complexity of the antioxidants and their mechanism of actions, no single testing method is capable of providing a antioxidant profile of a studied sample and a combination of different methods is necessary. Another limitation of DPPH method was the false negative reaction with the SH-group compounds, such as Glutathione (GSH). Despite such limitations, DPPH free radical scavenging assay can be helpful for primary screening and finding of novel antioxidants. In the methods of estimation of analgesic and anti-inflammatory activity, various Non-steroidal anti-inflammatory drugs are used. Though these drugs are effective in controlling pain, their wide range of adverse effects are the biggest limitations in their use. About 34-46% of the users of NSAIDs usually sustain some gastrointestinal damage due to the inhibition of the protective cyclo-oxygenase enzyme in gastric mucosa.

The triterpenoids isolated from various species of cucurbita family have been reported to possess anti-inflammatory activities. The cucurbitacins from *Lagenaria siceraria* were evaluated for their anti-inflammatory and inhibitory effects on the growth of cancer cell lines. Cucurbitacin glucosides are reported to possess antioxidant and free-radical scavenging activities. Thus the triterpenoids might be responsible for the free radical scavenging, analgesic and anti-inflammatory activity. Thus, the extract of *Lagenaria siceraria* seeds can be employed as antioxidant, analgesic, anti-inflammatory agent for human body.

CONCLUSION

The present study on extract of *Lagenaria siceraria* has demonstrated that this plant has significant analgesic and anti-inflammatory properties and it justifies the traditional use of this plant in the treatment of various types of pains and inflammations.

ACKNOWLEDGMENTS

The authors are highly thankful to Professor A.C. Rana and all faculty members of Rayat Institute of Pharmacy for their encouragement and support. We are also grateful to Rayat and Bahra Educational and Research Trust for their unconditional help to carry out this project.

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