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PHYTOCHEMICAL SCREENING AND EVALUATION OF VARIOUS EXTRACTS OF *LAGENERIA SICERARIA* FOR ANTIOXIDANT ACTIVITY

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ABSTRACT: *Lagenaria siceraria* was commonly known as Bottle gourd Syn. Doodhi, Syn. Lauki (Hindi) Kadoo (Marathi) which is official in Ayurvedic Pharmacopoeia. Preliminary phytochemical screening of the crude extracts revealed the presence of carbohydrates, proteins, *etc*. and secondary metabolites like saponins, glycosides, alkaloids, *etc*. in all the extracts but tannins were found absent in ethanolic and pet. ether extract. The defeated powdered material was further used for extraction with different organic solvents, namely: aqueous, ethanol, and pet. ether. There was a significant increase in the percentage of radical scavenging activity of ethanolic extract with an increase in concentration, followed by aqueous then pet. ether extracts. But % RSA of ethanolic extract was lesser than that of ascorbic acid.

INTRODUCTION: Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Natural products from plant, animal, and minerals have been the basis of the treatment of human disease [1]. Today estimate that about 80% of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathic use several plant species to treat different ailments. The use of herbal medicine is becoming popular due to toxicity and side effects of allopathic medicines. India has one of the richest plants medical traditions in the world [2].

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There are estimated to be around 25,000 effective plant-based formulations, used in folk medicine and known to rural communities in India. There are over 1.5 million practitioners of the traditional medicinal system using medicinal plants in preventive, promotional and curative applications. It is estimated that there are over 7800 medicinal drug-manufacturing units in India, which consume about 2000 tonnes of herbs annually [3]. Exploration of the chemical constituents of the plants and pharmacological screening may provide us the basis for developing the leads for the development of novel agents.

Also, herbs have provided us with some of the very important life-saving drugs used in the armamentarium of beta damaging to the human body than synthetic drugs. Therefore laboratories around the world are engaged in the screening of plants for biological activities with therapeutic potential. The market for Ayurvedic medicines is estimated to be expanding at 20% annually. Sales of medicinal plants have grown by nearly 25% in

India in the past ten years (1987-96), the highest rate of growth in the world. But the per capita expenditure in India on medicines per annum is among the lowest in the world. In other developing countries too, plants are the main source of medicine. Two of the largest users of medicinal plants are China and India [4]. Traditional Chinese Medicine uses over 5000 plant species; India uses about 7000.

According to Export-Import Bank, the international market for medicinal plant-related trade is having a growth rate of 7% per annum. China's share in world herbal market is US$ 6 billion while India's share is only US$1 billion. The annual export of medicinal plants from India is valued at Rs. 1200 million. All the major herbal-based pharmaceutical companies are showing a constant growth of about 15 percent. Traditional medicine has served as a source of alternative medicine, new pharma-ceuticals, and healthcare products.

Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmaco-logically active compounds. The derivatives of medicinal plants are non-narcotic with little or no side effects [5].

***Lagenaria siceraria*:** Cucurbitaceae family is commonly mentioned as the gourd, melon or, pumpkin family, is medium sized generally a climbing plants family, composing 118 genera and 825 species having wide distribution.

Taxonomical Classification:

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Cucurbitales
Family	:	Cucurbitaceae
Genus	:	Lagenaria
Species	:	*L. siceraria*

Among all plants of the Cucurbitaceae family, *Lagenaria species* have an important contribution to the overall popularity. The bottle gourd belongs to genus *Lagenaria* that is derived from the word lagena, meaning bottle. In the older literature, it is often referred to as *Lagenaria vulgaris* (Common), or *Lagenaria leucantha* (White flowered gourd) but it is now generally agreed that the correct name is *Lagenaria siceraria* (Mol.) Standl. *Lageneria siceraria* commonly known as Bottle gourd Syn. Doodhi, Syn. Lauki (Hindi), Kadoo (Marathi) which is official in Ayurvedic Pharmacopoeia [6].

Part Used: Fruit, root, leaves and seed oil.

Lagenaria is a large pubescent, climbing or trailing herb with stout 5- angled stems and bifid tendrils, found throughout India, either wild or cultivated. Leaves are long, petioled, 3-5 lobed, 7-10* 10-12 cm, hirsute; Fruits are large, up to 1.8 m. Long, fruit bottle shaped with a hard shell- like epicarp when ripe; numerous seeds, long, white, smooth, 1.6- 2.0 cm long, horizontally compressed with marginal groove 2.

Flowers are white, solitary, axillary unisexual. Male flowers possess a botanical description of the calyx and campanulate, tube narrow, lobes 5, linear; petals 5, free, white; stamens 3, Female flowers possess botanical description of calyx and corolla as in male flowers. overy densely villous, style thick, stigmas 3, bilobed 2.[7,8]

Chemical Composition:

Analysis of Edible Portion of the Fruit Gave Following Values: Moisture, 96.3; protein, 0.2; fat (ether extract), 0.1; carbohydrates 2.9; mineral matter 0.5; calcium 0.02; and phosphorus <0.01%. Other mineral elements reported to be present are: iron (0.7 mg/100g.), sodium (11.0 mg/100g)., potassium (86.0 mg/100g.) and iodine (4.5 mcg/ kg.). Glucose and fructose have been detected.

The amino acid composition of the fruit is as follows: leucines 0.8; phenylalanine 0.9; valine 0.3; tyrosine 0.4; alanine 0.5; threonine 0.2; glutamic acid 0.3; serine 0.6; aspartic acid 1.9; cystine 0.6; cysteine 0.3; arginine 0.4; and proline 0.3mg/g [9].

Fruit: Fruit is a good source of B vitamins and a fair source of ascorbic acid. Bitter fruits yield 0.013% of solid foam containing cucurbitacins B, D, G and H, mainly cucurbitacin B; these bitter principles are present in the fruit as aglycones. Leaves contain cucurbitacin B, and roots, cucurbitacins B, Dand E. Phytochemical screening

of the fruit revealed two steroids were isolated from the petroleum ether fraction, and they were identified as fucosterol and campesterol. Sugar and phenolic content of the fresh product was assayed, providing a partial nutritional characterization of this vegetable. Glucose and fructose (about 1:1 ratio) and traces of sucrose were found; also, a small amount of unidentified mono- and dicaffeoylquinic acid derivatives were detected [10].

Flavonoid complexes occurring in the medicinal plants *Lagenaria siceraria* were found to be flavone Glycosides 20 Four new D:C-friedooleanane-type triterpenes isolated, 3b -O-(E)-feruloyl- D:Cfriedooleana-7,9(11)-dien-29-ol, 3b -O-(E)- coumaroyl-D: Cfriedooleana-7, 9(11)-dien-29-ol, 3b-O- (E)-coumaroyl-D: Cfriedooleana-7, 9(11) –dien -29 -oic acid, and methyl 2b, 3b -dihydroxy-D:C-friedoolean- 8-en-29-oate. A water-soluble polysaccharide, isolated from fruiting bodies of *Lagenaria siceraria,* is composed of methyl-á-d-galacturonate, 3-O-acetyl methyl-á-d-galacturonate, and â-d-galactose in a ratio of nearly 1:1:1. This polysaccharide showed cytotoxic activity *in-vitro* against human breast adeno-carcinoma cell line (MCF-7).

Seeds: The seeds considered as the least important are having prime role in the human nutrition due to encapsulation of innumerable phytochemicals, vitamins, minerals, amino acid along with saponin and essential fixed oils especially of unsaturated type Seeds are reported to contain saponin. Analysis of seed kernals (68% of seed wt.) gives following values: moisture, 2.47; protein,30.72; oil,52.54; carbohydrates, 8.3; fiber, 1.58; ash, 4.43; CaO,0.11; and P_2O_3, 2.46%.

The oil obtained from seed kernels is clear and pale yellow. Kernels from ripe seeds gave 45% of oil with the following characteristics: n40d, 1.4711; sap. equiv., 301.6; iodine value, 126.5; free fatty acids, 0.54%; and unsaponified matter, 0.67%. The components of free fatty acids are linoleic acids 64.0; oleic, 18.2; and saturated fatty acids, 17.8%. Seeds are reported to contain Lagenin [11].

MATERIAL AND METHOD:

Collection of Plant Material and Authentication: The fruit was obtained from the vegetable market of Durg. Dr. Mrs authenticated them. Ranjana Shrivastav, Professor (Botany) and HOD, Govt. V.Y.T Autonomous P.G. College, Durg (C.G)

Preparation of Sample: The seeds were isolated fruit and were dried in the shade for 12 days after complete drying of seeds, they were powdered coarsely using a grinder and accurately weighed 125 g of powder was defeated using Petroleum ether to remove the fats from the seeds. The defeated powdered material was further used for extraction with different organic solvents, namely: aqueous, ethanol, and pet. ether.

Extraction Method: Solvents used for extraction were of Lab. Grade. Extraction was carried out using Soxhlet apparatus. It was originally designed for the extraction of a lipid from a solid material.

However, a Soxhlet extractor is not limited to the extraction of lipids. It is a continuous heating Extraction method. The solvent is heated to reflux. The solvent vapour travels up distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber [12].

Phytochemical Screening: Phytochemical screening of the extract was carried out to identify primary metabolites like carbohydrates (Molisch reagent test), Proteins (Biuret test) and of secondary metabolites such as alkaloids (Mayer's test), flavonoids, terpenoids (Salkowski test), tannins (Ferric chloride test), saponins (Frothing test), cardiac glycosides (Keller-Killiani test) and anthraquinones (Borntrager's test) [13].

***In-vitro* Antioxidant Assay:** The antioxidant activity of different plant extracts was carried out using the *in-vitro* method DPPH free radical scavenging assay. DPPH reagent was purchased from Sigma Company (Kolkata). Four ml of 0.004 % of DPPH- methanolic solution was added in each of the test tubes containing 1000µl of extracts of different concentration.

The mixture was shaken vigorously and incubated for 30 min at room temperature. The absorbance of the resulting solution was measured at 517 nm UV-Visible Spectrophotometer [14]. Blank was prepared with 4 ml of DPPH -methanolic solution (without

extract). A positive control was taken as ascorbic acid (standard).

Percentage of DPPH scavenging activity determined as follows:

% DPPH radical-scavenging = The absorbance of control – Absorbance of Sample / Absorbance of control × 100

RESULTS:

Extractive Value: Soxhlet apparatus was used for extraction of *L. siceraria* seeds using different solvents. The extractive value was found more in ethanolic extract followed by aqueous and then by petroleum ether **Table 1**.

TABLE 1: EXTRACTIVE VALUE OF *L. SICERARIA* SEEDS IN DIFFERENT SOLVENTS

S. no.	Extract	% Yield (w/w)
1.	Pet.Ether	0.60
2.	Ethanolic	2.2
3.	Aqueous	3.50

Phytochemical Screening: Preliminary phyto-chemical screening of the crude extracts revealed the presence of carbohydrates, proteins *etc.* and secondary metabolites like saponins, glycosides, alkaloids, etc. in all the extracts but tannins were found absent in ethanolic and pet. ether extract **Table 2**.

TABLE 2: PHYTOCHEMICAL SCREENING RESULTS OF DIFFERENT EXTRACTS OF *L.SICERARIA*

S. no.	Metabolites	Pet. Ether	Ethanolic	Aqueous
1	Carbohydrates	+	+	+
2	Proteins	+	+	+
3	Alkaloids	+	+	+
4	Glycosides			
5	Flavonoid	+	+	+
6	Saponin	+	+	+

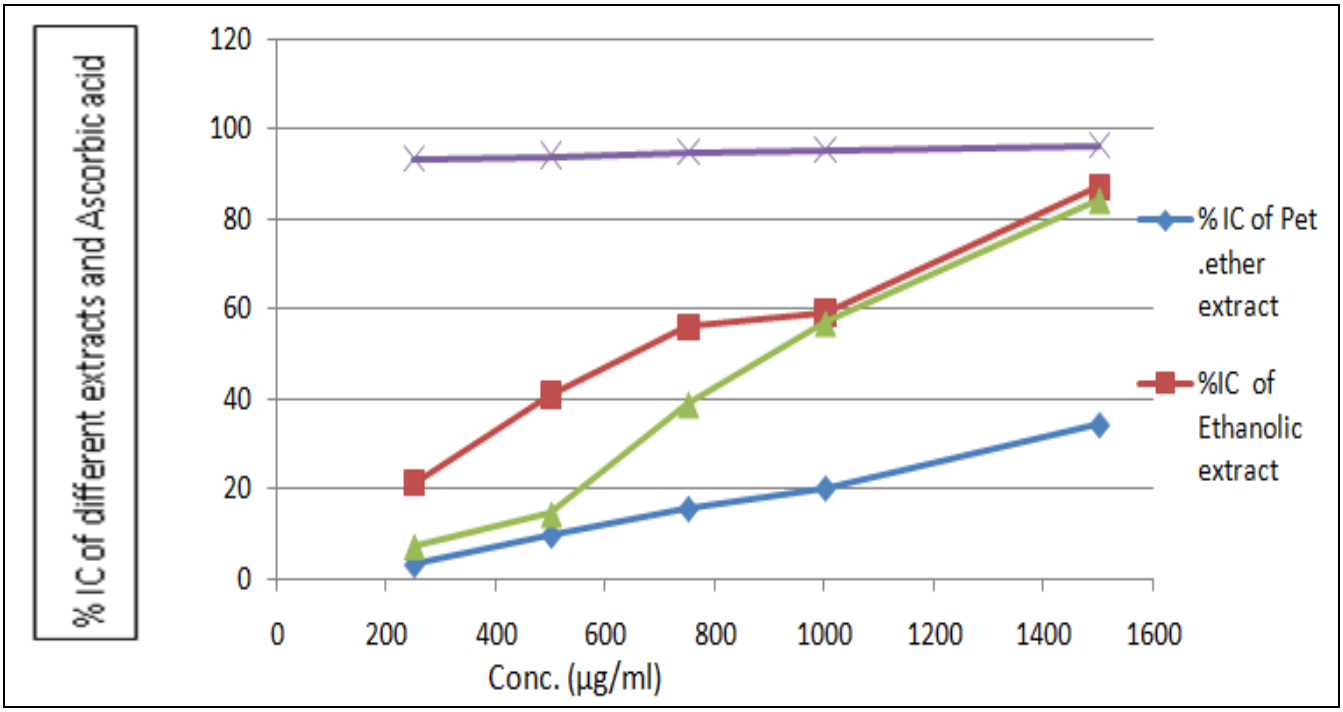

FIG. 1: GRAPHICAL COMPARISION OF % INHIBITION CONCENTRATION OF DIFFERENT EXTRACTS OF *LAGENARIA SICERARIA* SEEDS AND ASCORBIC ACID

***In-vitro* Antioxidant Assay:** The scavenging effect of petroleum ether, ethanolic, and aqueous of *Lagenaria siceraria*. The comparison of % inhibition concentration of different extracts with that of the standard were presented graphically in **Fig. 1**. There was a significant increase in the percentage of radical scavenging activity of ethanolic extract with an increase in concentration, followed by aqueous then pet. ether extracts. But % RSA of ethanolic extract was lesser than that of ascorbic acid [15, 16].

DISCUSSION: The phytochemical screening of different extracts of *Lagenaria siceraria* revealed that it contained chemicals like flavanoid which is claimed to have antioxidant response. The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search for the *in-vitro* general antioxidant activity of pure compounds as well as plant extracts [17, 18].

The decrease in absorbance by the DPPH radical with an increase in the concentration of the extract which manifested in the rapid discoloration of the purple DPPH, suggest that ethanolic extract of *Lagenaria siceraria* seeds has antioxidant activity due to its proton donating ability [19].

CONCLUSION: It is concluded that as from this study antioxidant potential of *L. siceraria* seeds extract is illustrated. Therefore, it is necessary to exploit its maximum potential in the field of Medicinal and pharmaceutical sciences for novel and fruitful application as antioxidant activity.

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