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Phytochemical Screening and Pharmacological Activity of Seed Extract and Seed Oil of *Lagenaria siceraria*

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Abstract: The present study includes detailed aspects of seeds of *Lagenaria siceraria (*Mol.) Standley used as medicinal plant. The *Lagenaria* seeds contain steroidal moieties like avenasterol, codisterol, elesterol, isofucasterol, stigmasterol and Legenin, a ribosome inactivating protein(RIP) isolated from seed shows various pharmacological activity. Antioxidants consist a group of vitamins, minerals and enzymes that have health enhancing effects. Antioxidants terminate the chain reactions by removing free radical intermediates before they do harm to our bodies. Antibacterial agents are inhibitory chemicals employed to kill micro-organisms or prevent their growth.Antianxiety agent a pyschotropic medication involves functional category of drugs useful in the treatment of anxiety and able to reduce anxiety at dosage that not cause excessive sedation.The elevated plus maze is a widely used behavioral assay for rodents and it has been validated to assess the anti-anxiety effects of pharmacological agents.An increase in open arm activity reflects anti-anxiety behavior.

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INTRODUCTION:

Lagenaria siceraria Standley is commonly known as bottle-gourd. Fresh Fruit of obtained from *Lagenaria siceraria* (Molina) belonging to family cucurbitaceae (Nigam et al., 2015). Fruits (shown in figure 1) contain carbohydrate, protein, fat (ether extract), fibers, mineral matter, calcium and phosphorous.The seeds contain steroidal moieties like avenasterol, codisterol, elesterol, isofucasterol, stigmasterol, sitosterol, compesterol, spinasterol. it used as a cardiotonic, liver tonic, anti-inflammatory, diuretic agent (Seth et.al.,2010), analgesic, anti-inflammatory activity, antioxidant activity (Deshpande et.al.2008). Seed containg Legenin, a ribosome inactivating protein (RIP) isolated from seed shows antitumor, anti HIV, anti proliferative, immune protactive properties (Pradhan et.al.2013).

Fig 1: Fruit of *Lagenaria siceraria*

Antioxidant is a molecule capable of inhibiting the oxidation of molecules. Oxidation reactions produce free radicals and these radicals can starts chain reactions. When the chain reaction occurs in a cell, it causes damage or death to the cell (James et.al.2003). Antioxidants consist of a group of vitamins, minerals and enzymes having health enhancing effects for our bodies. Antioxidant works to neutralize the free radicals before they harm to our bodies. Free radicals are formed by cells being exposed to a variety of substances such as radiation, chemicals, pollution, smoke, drugs, alcohol, pesticides and sun and through various metabolic processes (Evelson et.al.2001).

The agent used as the treatment of diseases inhibitory chemicals employed to kill micro-organisms or prevent their growth, are called antimicrobial agents (Qaralleh et al., 2009; Majali et al., 2015). These are classified according to their application and spectrum of activity. germicides act as viricides (killing viruses), bacteriocides (killing bacteria), algicides (killing algae) or fungicides (killing fungi) (Gibbons et.al. 2004). antibacterial agents are classified in three categories:

- Antibiotics and chemically synthesized chemotherapeutic agents
- Non-antibiotic chemotherapeutic agents
- Immunological products (Somchit et.al. 2003)

Anxiety is a feeling of fear, uneasiness,worry and unfocused as an overreaction to a situation (American Psychiatric Association, 2013). It is accompanied by muscular tension, restlessness, fatigue and problems in concentration (Bouras et.al. 2007). Anti·anxiety agents are functional category of drugs useful in the treatment of anxiety and able to reduce anxiety at doses that do not cause excessive sedation. The outcomes of anxiety are highlighted in figure 2.

Radial Arm Maze has been described as a simple method for assessing anxiety responses of rodents (Alicia et.al.2007). The radial arm maze designed by Olton and Samuelson in 1976.The apparatus consists of eight equidistantly spaced four open and four enclosed arms (Iton et.al. 1976, Cole et.al. 2003). Anxiety reduction in the plus-maze is indicated by an increase in the proportion of time spent in the open arms and an increase in the proportion of entries into the open arms (Hogg et.al. 1996).

MATERIAL AND METHODS:

1. Collection of plant materials:

The seeds of *Lagenaria siceraria* (cucurbitaceae) were purchased from the market of Raipur & Bhilai. They were cleaned with tap water for removal of adhering dirt and soil and powderd it. (Shown in figure 3 and 4)

Fig 3: Marketed product of dry seed of *Lagenaria siceraria*

Fig 4 : Powdered dry seed

2. Preparation of Extract:

The fresh and crushed dry seeds, were subjected to different successive soxhlet extraction respectively. Extraction was done by using following solvents:

a) Ethanol
b) Combination of Ether and Ethanol
c) Combination of Ether and Acetone

Fig 5: Soxhlet Apparatus

3. Separation of Oil: The oil obtained in the alcoholic extract was separated by using separating funnel. The separation is shown in figure 6. The yield of oil was found to be 4.78 gm.

Fig 6: Oil Separation

4. Preliminary Phytochemical Screening:

The seed extract was subjected to different qualitative chemical tests for detection of various phytoconstituents present in the extract (Dr. Khandelwal et.al. 2006, Dr. Kokate et.al. 2006). The details are enlisted in table 1.

5. Antioxidant activity by DPPH assay

The hydrogen atoms donation ability of polyphenol-rich extract was measured from the bleaching of purple colored methanol or ethanol solution of DPPH. The stable radical 1,1-Diphenyl- 2-picrylhydrazyl (DPPH) uses in this spectrophotometric method as a reagent (21, 22).4 ml of different concentration of the extracts dissolved in methanol or ethanol were added to 2.5 mL of a DPPH solution (0.004% solution using 20mg DPPH with 500 ml of ethanol). After 30 min incubation period at room temperature the absorbance was recorded against a blank at 515 nm (Susithra et.al. 2011; Althunibat et al., 2013).

Formula for determination of antioxidant

I% = (Ao – As/ Ao)×100

Where, I = Percentage inhibition DPPH activity.
Ao = Absorbance of the blank solution (DPPH solution)
As = Absorbance of the test compound.

6. Pharmacological study

For pharmacological activity of seeds extract on wister rats were used.

Animal used: Rats are collected from the animal house of SRIP Kumhari, Durg (C.G.). The rats were divided in two groups and each groups contain six rats.
Group I - control
Group II - dry seed extract
All the protocol of the study have been approved by Institutional Animal Ethical Committee.

Procedure: Before starting the experiment all rats were kept in fasting condition. After 24 hrs the weight of all rats were noted. Take water (1 ml) as control and dry seed extract (1^{st} four days-0.2 ml and next 6 days-0.5 ml) feeded on rats of 1^{st} and 2^{nd} groups respectively by using feeding needle. After 1 hr. observed the action of rats on radial arm maze either they are in closed arm or in open arm at time interval of 5 min. and note down the time remaining inside the arm. Repeat the steps for next 5 days. Compare the result.

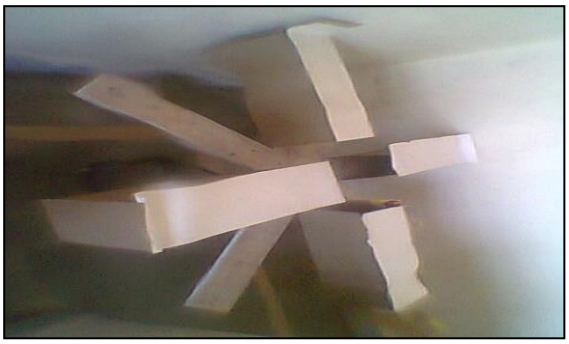

Fig 7: Simple Home Made Eight Arm Radial Maze

7. Antibacterial activity of *Lagenaria siceraria* seed oil :

Antibacterial activity evaluated by using cup plate method on nutrient agar medium. This was confirmed by inhibitory effect on bacterial growth as reflected by the inhibition zone, compared to that of known antibiotics. The sterile nutrient agar medium (20 ml) in the petri dishes was uniformly smeared using sterile cotton swabs with test cultures of human pathogenic bacteria E. coli, pseudomonas and staphylococus. The nutrient agar media prepared by dissolving 10 g beef extract, 5g starch, 10g peptone, 5g NaCl and 5g agar in 1 litre distilled water. The inoculums prepared from fresh overnight broth culture in nutrient broth .The wells of 5 mm diameter were made by using a sterile cork borer in each petri dish and the standard antibiotic solution 10mg/ml, *Lagenaria siceraria* seed oil of conc. 100mg/ml and 200mg/ml were added, a blank well loaded without test compound was regarded as control. Plates were incubated at 37°C for 24 h and the resulting zone of inhibition was measured by comparing control and the standard antibiotic (Mylarappa et al., 2010, Majali et al., 2015).

RESULTS AND DISCUSSION:

1. Phytochemical Screening

The phytochemical screening of seeds extract of *Lagenaria siceraria* (cucurbitaceae) revealed the presence of carbohydrates, reducing sugar, cardiac glycosides, flavonoids and fixed oil. However polysaccharides, proteins, steroids, tannins and steroids were absent in the extract. The observation table and inference is shown in table 1.

Table 1: List of Phytochemical Test of various phytoconstituents

S. No.	Name of Phytoconstituents	Name of Test
1.	Test for Carbohydrates	Molish's test
2.	Test for Reducing Sugars	Fehling's test
		Benedict' test
3.	Test for Monosaccharides	Barford's test
4.	Test for Hexose Sugars	Selwinoff's test
5.	Test for Non-Reducing Polysaccharides(Starch)	Iodine test
		Tannic acid test for starch
6.	Test for Proteins	Biuret test
		Million test
7.	Test for Amino acids	Ninhydrin test
8.	Test for Steroids	Salkowski reaction
		Libermann-Burchad reaction
9.	Test for Cardiac glycosides	Legal's test
10.	Test for Saponin glycosides	Foam test
11.	Test for Flavanoids	
12	Test for Alkaloids	Mayer's test
		Hager's test
		Wagner's test
13.	Test for Tannins and Phenolic Compounds	5% $FeCl_3$ Solution
		Lead Acetate Solution
		Gelatin Solution
		Bromine Water
		Acetic acid solution
		Dil HNO_3 acid
14.	Test for fixed oil	Using Sodium Hydroxide
		Using Sodium Hydrogen Sulphate

Table 1: Phytochemical Screening of Fresh & Dry seed Extract

S. No.	Name of Chemical Test	Observation
1.	**Test for Carbohydrates**	
	Molish's test	+
2.	**Test for Reducing Sugars**	
	Fehling's test	+
	Benedict' test	+
3.	**Test for Monosaccharides**	
	Barford's test	+
4.	**Test for Hexose Sugars**	
	Selwinoff's test	-
5.	**Test for Non-Reducing Polysaccharides(Starch)**	
	Iodone test	-
	Tannic acid test for starch	-
	Test for Gum	-
6.	**Test for Proteins**	
	Biuret test	-
	Million test	-
7.	**Test for Amino acids**	
	Ninhydrin test	-
	Tests for volatile oils	-
8.	**Test for Steroids**	
	Salkowski reaction	-
	Libermann-Burchad reaction	-
9.	**Test for Cardiac glycosides**	
	Legal's test	+
10.	**Test for Saponin glycosides**	
	Foam test	-
11.	**Test for Flavanoids**	+
12	**Test for Alkaloids**	
	Mayer's test	-
	Hager's test	-
	Wagner's test	-
13.	**Test for Tannins and Phenolic Compounds**	
	5% $FeCl_3$ Solution	-
	Lead Acetate Solution	-
	Gelatin Solution	-
	Bromine Water	-
	Acetic acid solution	-
	Dil HNO_3 acid	-
14.	**Test of fixed oil**	
	Using Sodium Hydroxide	+
	Using Sodium Hydrogen Sulphate	+

2. Antioxidant activity [DPPH Assay]

Table 2.1-Ascorbic Acid (Standard)

S.No.	Conc.ug/ml	% inhibition
1	25	93.5%
2	75	94.7 %
3.	125	99.1

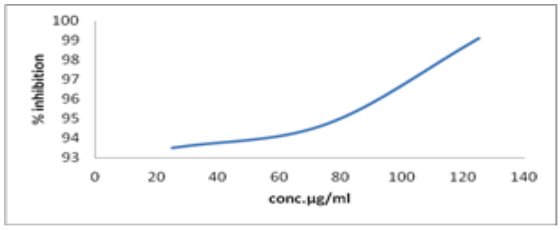

Fig 7: Standard curve of Ascorbic Acid

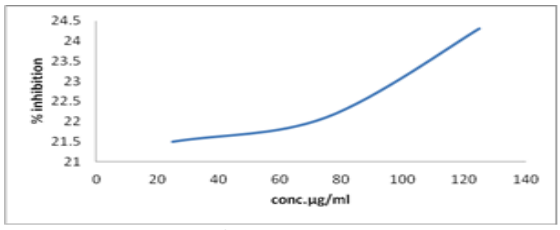

Fig 8: Standard curve of Fresh Seed Alcoholic extract

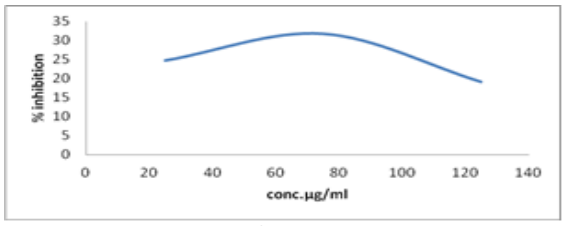

Fig 9: Standard Curve of Fresh Seed Ether +Alcoholic extract

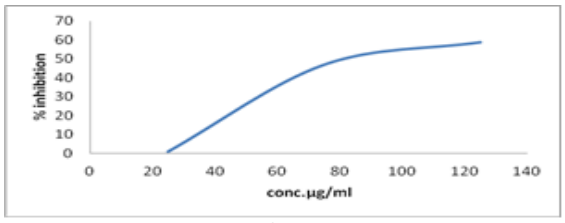

Fig 10: Standard Curve of Fresh Seed Ether +Acetone extract

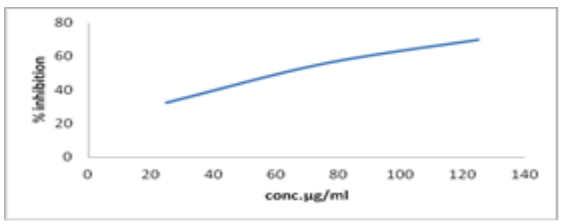

Fig 11: Standard curve of Dry Seed Alcoholic extract

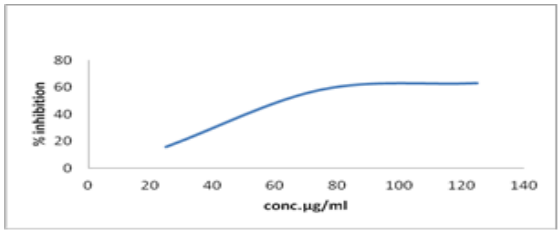

Fig 12: Standard curve of Dry Seed Ether +Alcoholic extract

Table 2.2-Fresh Seed Extract

S.No.	Conc ug/ml	% inhibition		
		A	B	C
1	25	21.5 %	24.7 %	1.02 %
2	75	22.16 %	31.7 %	47.18%
3	125	24.31 %	19.14 %	59.0 %

A=alcoholic extract, B=ether+alcoholic extract, C=ether+acetone extract

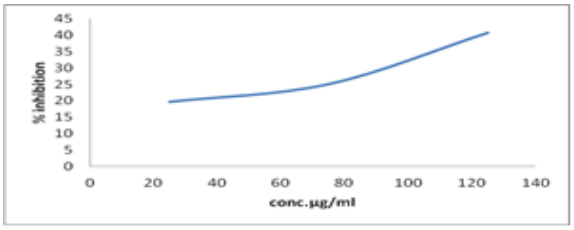

Fig 13: Standard curve of Dry Seed Ether +Acetone extract

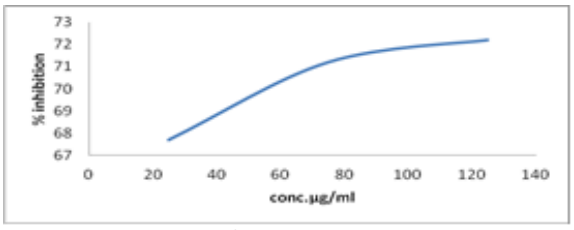

Fig 14: Standard curve of Dry Seed Oil

Table 2.3-Dry Powder Seed Extract

S.No.	Conc.ug/ml	%inhibition		
		A	B	C
1	25	32.5 %	16.04 %	19.6 9%
2	75	55.6 %	58.18%	25.04 %
3	125	70.0%	62.94 %	40.79 %

A=alcoholic extract, B=ether+alcoholic extract, C=ether+acetone extract

3. Antibacterial Activity of seed oil

Figure 15: Inhibition of E.coli

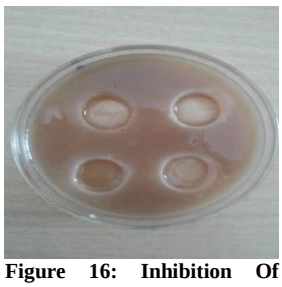

Figure 16: Inhibition Of Pseudomonas

Table 3.1- Inhibitory Effect Of E.coli

S.NO.	Type of sample	Zone of inhibition	Activity
1.	Control (ethanol)	-	No activity
2.	Standard(azithromicine)	2 mm	Good
3.	Sample 100mg/ml	4mm	Moderate
4.	Sample 200mg/ml	5 mm	Very good

Table 3.2- Inhibitory Effect Of Pseudomonas

S.NO.	Type of sample	Zone of inhibition	Activity
1.	Control (ethanol)	-	No activity
2.	Standard(azithromicine)	3 mm	Very Good
3.	Sample 100mg/ml	2 mm	Moderate
4.	Sample 200mg/ml	1 mm	Good

4.Pharmacological activity on rat done by using radial -maze

Table-4.1

Day-1	Average Weight (g)	Average Time in closed arm (sec)
Control	106	288
Dry Seed extract	100	278

Table-4.2

Day-2	Average Weight (g)	Average Time in closed arm (sec)
Control	110	290
Dry Seed extract	93.3	276

Table-4.3

DAY-3	Average Weight (g)	Average Time in closed arm (sec)
Control	113.3	287
Seed extract	102	282

Table-4.4

Day-4	Average Weight (g)	Average Time in closed arm (sec)
Control	108.6	290
Seed extract	96.6	287

Table-4.5

Day-5	Average Weight (g)	Average Time in closed arm (sec)
Control	106.6 94	290
Seed extract	95.3	286

Table-4.6

Day-6	Average Weight (g)	Average Time in closed arm (sec)
Control	103.3	281.3
Seed extract	91.3	264

The Seed extract shows decreses in weight of rats and time in the closed arms of radial –maze as compared to control.

CONCLUSION

Based on extensive experimental work done, it can be concluded the fresh and dry Seed Extract of *Lagenaria siceraria* shows the presence of carbohydrates, cardiac glycoside, Flavanoids and fixed oil. Dry Seed Extract and seed oil shows maximum antioxidant activity. Decreased in weight of rats and decreased the average time inside the closed arms of radial maze model thus indicating the dry Seed Extract shows anti-anxiety activity. The seed oil of *Lagenaria* exhibited good antibacterial activity against E.coli and Staphylococcus.

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