Innovations

Phytochemical Investigation and Antioxidant Activity of Ximenia Americana and Lindera Communis

Priyanka Sabale[1*] Rajasekaran.S[2]

1. Department of Pharmacology, Research scholar, Institute of Pharmaceutical sciences and Research centre, Bhagwant University, Sikar Road, Ajmer, Rajasthan, India-305004.

2. Department of Pharmacology, Institute of Pharmaceutical sciences and Research Centre, Bhagwant University, Sikar Road, Ajmer, Rajasthan, India-305004

***Corresponding author: Priyanka Sabale**

Abstract:

The Olacaceae and Lauraceae families are home to the genera Ximenia americana and Lindera communis. Traditional medicine makes extensive use of the herbs Ximenia americana and Lindera. Particularly notable phytochemicals include those found in the leaves, fruits, and roots of Lindera plants as well as the fruit of Ximenia americana. These phytochemicals include alkaloids, anthraquinones, cardiac glycosides, flavonoids, glycosides, phenolic compounds, phlobatannins, quinones, saponins, tannins, and terpenoids. It is mostly found in tropical areas of Africa, Brazil, Brazil, India, New Zealand, Central America, and South America. A number of biological activities, including those that are antimicrobial, antifungal, anticancer, antineoplastic, antitrypanosomal, antirheumatic, antioxidant, analgesic, moluscicide, pesticidal, and that have hepatic and heamatological effects, have been demonstrated by the constituents of Ximenia americana and Lindera communis, according to studies conducted in recent years. We come to the conclusion that the plants Ximenia Americana and Lindera communis had potential therapeutic benefit and were significant in elaborating the different pharmacological actions, but the many traditional claims were not supported by scientific evidence.

Key words: *Ximenia americana, Lindera communis, Phytochemical constituents, Antioxidant activity.*

Introduction:

Ximenia americana[1] is one of the Olacaceae family edible wild fruits[2,3]. The fruit's medicinal value makes it suitable for novel medication development. In general, Africa, India, New Zealand, Central America, and South America are where you can get the fruit. The fruit potential of X. americana is well known in Ethiopia[4,5]. The fruit can be found throughout the nation, although the southern region dominates with its abundant supply. The fruit is generally referred to locally as inkoy. It is green in colour when it is first beginning to mature and turns yellow or red when it is fully ripe. The fruit is located as a spine-equipped bush. Several studies were conducted on the yellow-colored fruits of Ximenia americana growing in many nations to determine its nutritional, anti-nutritional, phytochemical, and therapeutic qualities[6,7]. These studies demonstrated that the fruit flesh of X. americana has a significant amount of total polyphenol, vitamin C, and free radical scavenging action. The seed is a promising raw material for

medical use since it contains significant levels of polyphenols and antioxidant activity in both the fruit flesh and the seed[8]. Contrary to other nations, Ethiopian X. americana fruit's yellow cultivar's flesh and seed have not yet been investigated for their phytochemicals, bioactive substances, and fatty acid profiles. The core genus Lindera, which belongs to the Lauraceae family and has more than 100 species, is a member of the Litseeae tribe. In particular, the tropical, subtropical, and temperate regions of Asia and North America are home to many plants of the Lindera genus. Because of its exquisite scent, plants from the genus Lindera are regarded as a rich source of essential oils and are frequently utilized in the manufacture of aromatic cosmetic items like soap and lubricants. Most notably, numerous Lindera plants have historically been employed in traditional medicine for their capacity to treat a variety of health-related issues, including pain, colds, urinary tract problems, rheumatoid arthritis, gastric ulcers, abdominal pain, cholera, and beriberi.

And therefore, this study was to assess the bioactive composition, free radical scavenging activity, and physicochemical properties of X. americana and Lindera communis.

Materials and Methods:

Extraction:

20 g from powdered plant sample of X. americana and Lindera communis were extracted with 200 ml water at 80 °C in a water bath for 12 hr. and then filtered. Water was evaporated using a lyophilizer. Residue was dissolved in sterile distilled water in order to obtain a final concentration of 100 mg/ml. All extracts were sterilized by filtering through a 0.22 μm filter (Millex). Plant materials, designation of treatments and yield (%) for aqueous extraction is 9.58%.

Preliminary phytochemical analysis

Preliminary phytochemical investigation was done with the coarse powder to detect the presence of carbohydrate, coumarins, flavonoids, steroid, quinone, tannin, and terpenoid in ethanolic extract.

Tests for alkaloids Dragendorff's test: Small amount of acetic acid and Dragendorff's reagent were applied to a couple of mg of Lindera communis Hemsl(L.) and Ximenia americana extract.extract dissolved in alcohol and Test for steroids

Liebermann-Burchard test: After dissolving the Lindera communis Hemsl(L.) and Ximenia americana extract in chloroform, 1 ml of acetic acid and 1 ml of acetic anhydride were applied, and the mixture was heated on a water bath and cooled. A few drops of concentrated sulfuric acid were applied to the sides of the test tube. The existence of steroids is shown by the appearance of a bluish green colour.

Salkowski's test: After dissolving the extract in chloroform, an equivalent amount of concentrated sulfuric acid was added. The presence of steroids is shown by the formation of bluish red to cherry red colour in the chloroform layer and green fluorescence in the acid layer.

Test for saponins: Small amount of Lindera communis Hemsl(L.) and Ximenia americana extract and distilled water was added. It wasshaken well. Appearance of stable froth formation indicates the presence of saponins.

Test for tannins: to small amount of Lindera communis Hemsl(L.) and Ximenia americana extract. A few drops of weak ferric chloride solution were added, and the development of a dark blue colour indicates the presence of tannins.

Test for Flavonoids: Shinoda's test: Small amount Lindera communis Hemsl(L.) and Ximenia americana extract.extract is dissolved in alcohol then is added with a few magnesium turnings and few drops of conc. hydrochloric acid and heated on a water bath. The existence of flavonoids is shown by the formation of a red to pink colour.

Test for phenol: To the Lindera communis Hemsl(L.) and Ximenia americana extract.extract in alcohol, added two drops of alcoholic ferric chloride. The presence of phenol is shown by the formation of a blue to black color.

Test for coumarins: A few drops of 2N sodium hydroxide solution is added to the alcohol extract of Lindera communis Hemsl(L.) and Ximenia americana extract. The presence of coumarins is shown by the formation of a dark yellow colour.

Test for triterpenoids: The Lindera communis Hemsl(L.) and Ximenia americana extract.extract was warmed with tiny bits and a few drops of thionyl chloride. The existence of triterpenoids is shown by the formation of pink colour.

Test for carboxylic acid: Lindera communis Hemsl(L.) and Ximenia americana extract.extract dissolved in water and is treated with sodium bicarbonate. Appearance of brisk effervescence indicates the presence of carboxylic acid.

Test for resin: Few mg of the Lindera communis Hemsl(L.) and Ximenia americana extract.extract was mixed with water and acetone. Turbidity indicates the presence of resin.

Test for quinone: A few mg of Lindera communis Hemsl(L.) and Ximenia americana extract.alcohol extract was treated with 0.5% of sodium hydroxide. The presence of quinone is shown by the presence of deep colours such as pink, purple, or red.

Extractive values

Alcohol soluble extractive: Weighed accurately about 4g of the Lindera communis Hemsl(L.) and Ximenia americana extract sample in a glass stoppered flask. Added 100 ml of distilled alcohol (approximately 95%). Shaked occasionally for about 6 hours. Allowed to stand for 18 hours. Filtrated quickly, taking care not to waste any solvent. Pipetted out about 25ml of the filtrate in a 100 ml pre-weighed beaker. On a water bath, it evaporated to dryness. It was kept on air oven at 105°C for 6 hours, cooled in desiccator for 30 minutes and weighed. The proportion of alcohol extractable matter in the sample was calculated. Repeated the experiment two times, and average value is obtained.

Water soluble extractive: Weighed accurately 4 g of the Lindera communis Hemsl(L.) and Ximenia americana extract. Sample in a glass stoppered flask. Added about 100 ml of distilled water, shacked well occasionally for about 6 hours. Allowed to stand for 18 hours. Filtered quickly, being careful not to lose any solvent. Pipetted about 25ml of the filtrate into a pre-weighed 100 ml beaker. Evaporated to dryness on a water bath. Kept it in an air oven at a temperature of 105°C for 6 hours. Cooled in a desiccator and weighed. The experiment was repeated twice, and the average value was obtained.

High performance thin layer chromatography (HPTLC): One gram of Lindera communis Hemsl (L.) and Ximenia americana extract powder was extracted with 10 ml of ethanol, 4, 8 and 12 µl of the above extract were applied on a pre-coated silica gel F254 on aluminium plates to a band width of 7 mm by using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (7:3). The formed plates were scanned at UV 254 and 366 nm before being derivatized with vanillin 58 sulfuric acid and scanned again at UV 254 and 366 nm. Rf, colour of the spots and densitometric scan were recorded.

Antoxidant Activity

Preparation of tissue homogenate: The excised liver tissue was washed with saline which is ice cold and stored at -20°C in deep freezer. Shortly before conducting the biochemical investigation the tissues were thawed and homogenized in phosphate buffer saline of pH 7.4, centrifuged at 4°C. The supernatant collected was subjected to assess catalase, glutathione peroxidase and lipid peroxidation using standard protocol. Excess supernatant of tissue homogenate was stored at -20°C for further use.

Determination of catalase activity: The catalase activity was determined by mixing 1mL of liver homogenate with 5ml of phosphate buffer, 4 mL of 0.2 M H2O2 in phosphate buffer and time was recorded. Exactly, After 3 minutes of H2O2 addition, a set of 1mL of the aforesaid reaction mixture was taken in 2mL dichromate acetic acid and then placed in a boiling water bath for about 10 minutes. The test tube containing reaction mixture was cooled under running water and reading was noted at 570nm against reagent blank using UV- visible spectrophotometer (Systronics 2201, India). Catalase activity in the liver tissue homogenate was expressed in micromoles H2O2 consumed/mg protein/minute.

Determination of glutathione peroxidase: The amounts of glutathione peroxidase in the liver homogenate were determined using standard protocol. The 0.2 ml of liver homogenate was added to the test tube containing 0.2 mL of EDTA, sodium azide, reduced glutathione, hydrogen peroxide were added, mixed thoroughly, and incubated at 37 ºC for 10 minutes. The reaction was stopped by adding 0.5 mL of TCA and centrifuged. 67 Supernatant about 0.5 mL was pipetted into test tube containing 4mL of disodium phosphate and 0.5mL of 5, 5-Dithiobis 2-nitrobenzoic acid (DTNB). The produced color was instantly read at 420nm using UV- visible spectrophotometer (Systronics 2201, India), and the standard was handled in the same way. The glutathione peroxidase level was expressed as micromoles of glutathione utilized /milligram protein/minutes at 370C.

Determination of lipid peroxidation: The lipid peroxidation in the liver homogenate was determined by measuring the MDA using thiobarbituric acid test. Liver homogenate (0.1 mL) was taken in a test tube containing 0.2 mL of 0.1% SDS, 1.5 mL of 20% acetic acid and 1.5 mL of 0.8 N aqueous solution of TBA. The volume of reaction mixture was made up to 4 ml by adding distilled water and heated at 950C for 60 minutes. The reaction mixture was allowed to cool and 1mL of distilled water, 5 mL of mixture of n-butanol and pyridine (15:1v/v) were added and shaken well. Finally it was centrifuged at 4000 rpm for 10min and read at 532 nm; absorbance was determined using UV- visible spectrophotometer (Systronics 2201, India). In the same way, the standard malondialdehyde was treated. The degree of lipid peroxidation was measured as µmoles of MDA formed /g of wet tissue.

Results:

Table 1: Preliminary Phytochemical screening of Lindera communis Hemsl (L.) and Ximenia americana – Aqueous Extract

Phytoconstituents	Aqueous Extract
Alkaloids	+
Glycosides	++
Carbohydrates	+
Flavonoids	+++
Saponins	++
Tannins	+++
Steroids	++
Proteins	-
Fats and oils	-

Preliminary phytochemical analysis of ethanolic Extract of Lindera communis Hemsl (L.) and Ximenia Americana – Ethanolic extract.

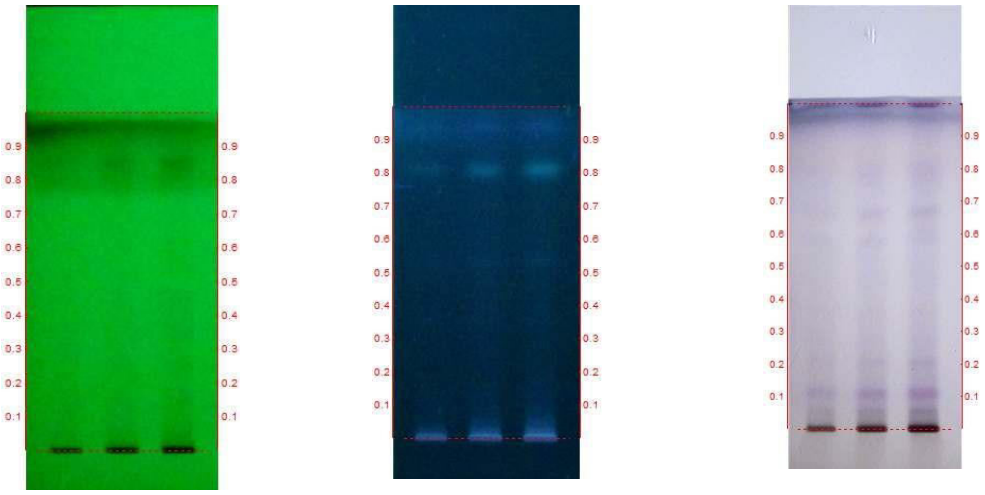
Preliminary phytochemical investigation was done with the coarse powder showed the presence of carbohydrate, coumarins, flavonoids, steroid, tannin, and terpenoid in ethanolic Extract Table 1- Preliminary phytochemical analysis of ethanolic Extract of Lindera communis Hemsl (L.) and Ximenia americana.

Table 2- Preliminary phytochemical analysis of ethanolic Extract of *Lindera communis Hemsl (L.) and Ximenia Americana* .

S.No	Tests	Colour	*Extract*
1	**Alkaloids**		
a	Dragandorf's test	Orange precipitate	Brown Colour Solution
b	Wagner's test	Red precipitate	Brown Colour Solution
c	Mayer's test	Dull white precipitate	Light yellow Colour
d	Hager's test	Yellow precipitate	Light yellow Colour
2	**Steroids**		
a	Liebermann-Burchard test	Bluish green	Green colour
b	Salkowski's test	Bluish red to cherry red	Red colour at junction
3	**Carbohydrate**		
a	Molish test	Violet ring	Violet ring
b	Fehling's test	Brick red precipitate	Brick red precipitate
c	Benedict's test	Red precipitate	Red precipitate
4	**Tannin**		
a	With $FeCl_3$	Dark blue or green or brown	Dark Green colour.
5	**Flavanoids**		
a	Shinoda's test	Red to pink	Pink Colour
6	**Saponins**		
a	With $NaHCO_3$	Stable froth	No froth
7	**Triterpenoids**		
a	Tin and thionyl chloride test	Pink	Pink Colour
8	**Coumarins**		
a	With 2N NaOH	Yellow	Yellow Colour Solution
9	**Phenols**		
a	With alcoholic ferric chloride	Blue to blue black,brown	Dark Green Colour
10	**Carboxylic acid**		
a	With water and $NaHCO_3$	Brisk effervescence	No brisk effervescence
11.	**Resin**		
a	With aqueous acetone	Turbidity	No Turbidity
12.	**Quinone**		
a	5% NaOH	Pink/purple/red	Dark Green Colour

HPTLC Fingerprinting

Figure 1.HPTLC photo documentation of Alcohol Extracts of Lindera communis Hemsl (L.) and Ximenia americana



At 254 nm At 366 nm After derivatisation

Table 3: Effect of ethanolic extract of *Lindera communis Hemsl (L.) and Ximenia americana* Extract on tissue parameters

S.no	Treatment	TBARS (mmol/g)	GSH (ug/g)	SOD (IU/dl)	Catalase (IU/dl)
1	Group I	29.8±0.7	67.1±5.2	409±35	54.2±0.71
2	Group II	108±8.0*	23.3±6.5**	160±6.3***	19.2±8.06***
3	Group III	33.2±3.6**	56.8±3.3*	171±11***	48.4±3.6***
4	Group IV	24±1.9***	55.3±3.1*	331±22***	48.2±1.9***

All values are expressed as mean ±SEM, one way Analysis of variance, followed by Dunnets,*p<0.05, **p<0.001, ***p<0.0001, ns-non significant when compared Group II with normal control and remaining groups with disease control.

oxidative stress parameters in Liver

Rifampicin elevated the levels of TBARS and reduced the levels of GSH, SOD and CAT significantly compared to control group. Treatment with extract of *Lindera communis Hemsl (L.) and Ximenia americana* at a dose of 200 mg/kg significantly reduced the levels of TBARS and no significant rise in levels of GSH, SOD and CAT levels. But the extract of doses 200 and 400 mg/kg decreased the levels of TBARS and increased the levels of SOD, GSH and CAT levels.

Thiobarbituric acid reactive substances

TBARS levels in Rifampicin induced group (108±8.0 nmol/gm wet.wt) were significantly high ($p<0.01$) compared to control (29.8±0.7nmol/gm wet wt). TBARS levels in low dose extract treated (33.2±3.6 nmol/gm wet wt) and high dose extract treated groups (24±1.9 nmol/gm wet wt) were significantly high ($p<0.001$) compared to disease control.

Reduced glutathione

GSH levels in Rifampicin treated group (23.3±6.5 µg/gm wet wt.) were significantly high ($p<0.001$) compared to normal control (67.1±5.2 µg/gm wet wt.). GSH levels in treated group ($p<0.05$), low dose extract treated group (56.8±3.3 µg/gm wet wt.) ($p<0.001$) showed significant increase compared to disease control.

Superoxide dismutase

SOD levels in Rifampicin induced group (160±6.3 IU/dl) had shown significant increase compared to control group (409±35 IU/dl) ($p<0.001$). High dose extract treated (331±22 IU/dl) ($p<0.0001$) showed significant increase in SOD levels compared to Rifampicin induced group i.e. disease control.

Catalase

Catalase levels in STZ induced group (19.2±8.06) has shown significant compared to normal control (54.2±0.71). High dose extract treated (48.2±1.9) group has shown significant increase compared to the disease control group.

Conclusion:

Our study gave experimental basis for the claim of hepatoprotective activity of Ethanolic extract of Lindera communis Hemsl (L.) and Ximenia americana. Preliminary Phytochemical analysis showed presence of carbohydrate, coumarins, flavonoids, steroid, tannin, and terpenoid. Flavonoids have potential anti-oxidant property. In vitro studies showed antioxidant effects.

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