Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Spl Issue [5] 2022 : 544-550

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Anti-Lipidemic Activity of Bark Extract-A Polyherbal Extraction and Phytochemistry Analysis

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ABSTRACT:
Hyperlipidemia is the greatest risk factor for coronary artery disease. Currently, allopathic antihyperlipidemic drugs are associated with a large number of side effects. The herbal treatment for hyperlipidemia has no side effects and is relatively despicable and locally available. The present work Cinnamomum verum belongs to family lauraceae and Azadirachta indica belongs to family Maleacea and Millatia pinnata belongs to family Fabaceae and this Samples of cinnamon bark collected from the local market. The barks of Azadirachta indica and Milletia pinnata were collected from the local area of Perambalur. Barks were thoroughly washed to remove dirt residues and oven dried over night at 70°C.Barkpowder(20g) was extracted in 200 ml 95% ethanol for 4-5 h in a Soxhlet extractor (4-6 cycles) until thesolventinthesiphontubebecamecolorless.Theextractwasfilteredandevaporatedtodrynessin vacuum on a rotary evaporator. Preliminary phytochemical analysis of the plant drug was carried out and it revealed the presence of Flavonoids, Tannins, Sterols, Quinones, Carboxyl groups,Saponin,Coumarin.Thin layer chromatography has been used as ananalytical tool, especially in organic chemistry, due to its high separation speed and its applicability to a large number of chemical compounds. The high sensitivity of the TLC is used to check the purity of the samples. The developed plates were air dried and then UV scanned at 275 nm. The lipase inhibitory activity of bark extracts was determined by measuring the hydrolysis of p-NPB to p-nitrophenol at 405 nm, Atorvastatin was used as a positive control (test concentration: 50–200µg/ml,). Cholesterol esterase inhibitory activity of bark extracts was performed using pancreatic lipase with minor modifications. The hemolytic effect of various extracts on human and rat erythrocytes was evaluated using washed erythrocytes (RBCs). Theresult revealed the Bark extract and have significant Anti lipidemic activity. The fact that the extract inhibited lipase and esterase at varying concentrations further supports the usefulness of the extract in decreasing cholesterol.
Keywords: *Azadirachta indica, Milletia pinnata,* UV, TLC, Anti- lipidemic, RBC.

Received 24.10.2022 Revised 21.11.2022 Accepted 22.12.2022

INTRODUCTION

Hyperlipidemia is the greatest risk factor for coronary artery disease. Currently, allopathic antihyperlipidemic drugs are associated with a large number of side effects.The herbal treatment for hyperlipidemia has no side effects and is relatively despicable and locally available. Medicinal plants are the "backbone" of traditional medicine and are considered a good source of life for all people due to their rich therapeutic properties and 100% naturalness. Medicinal plants are used by the majority of the population to treat various diseases and have a great impact on the world economy. Traditional therapeutic systems based mainly on plants, herbs and shrubs have always played a fundamental role in the global health system. Natural products are generally less toxic, have fewer side effects, and are readily available, increasing the need for herbal medicines. The subject is assigned to examine the botanicals for antihyperlipidemic activity and to use different models in this study we used bark of Cinnamon, Neem, Pongame Tree. Cinnamon is mainly used in flavor and essence industry for its fragrance, which can be incorporated into various types of foods, perfumes and medicines [3].

The main components of cinnamon are cinnamaldehyde and trans-cinnamaldehyde(Cin),which are present in the essential oil and thus contribute to the fragrance and the various biological activities observed in cinnamon [6].Cinnamon bark contains procyanidins and catechins.

The components of procyanidins include both procyanidin A-type and B-type linkages [1]. These procyanidins extracted from cinnamon and berries also possess antioxidant activities [5].The potential

effects observed in the use of Neem extracts can certainly attributed to cellular and molecular mechanisms, these mechanisms include free radical scavenging, detoxification, DNA repair,cell cycle alteration, mitigation of programmed cell death and autophagy,immune surveillance,anti-inflammatory, anti-angiogenic and antimetastatic activities and ability to modulate different signaling pathways [4]. , P. pinnata was used as a folk medicinal plant, mainly in the Indian medical systems of Ayurveda and Siddha. The antiinflammatory, antinonciceptive, antioxidant, antidiarrheal,antifungal, antiplasmodial, antiulcer, antihyperglycemic, antilipoxidative, antihypermmonic and analgesic functions are available in plant extracts. The tree is known for its multiple benefits and as a potential source of biodiesel. On average, the seeds are said to contain around 28-34% oil with a high proportion of polyunsaturated fatty acids[2].
Hyperlipidemia causes approximately 17 million deaths worldwide each year [1]. It is also an important factor in the development of cardiovascular disease and atherosclerosis. Atherosclerosis is a chronic inflammatory disease triggered by multiple factors, with the solid contribution of endothelial damage associated with lipid peroxidation. A plant-based diet rich in leaves, seeds, fruits, bulbs, vegetables, and legumes and low in saturated fat is an effective recipe for anyone suffering from severe atherosclerosis. The present study aimed to investigate the hypolipidemic potential of cassia cinnamon together with extracts from *Azadirachta indica and Milletiapinnata* to study the phytochemicals of bark extract qualitatively for detecting antilipase and esterase activity

MATERIALSANDMETHOD

Collection of bark samples:

Samples of cinnamon bark collected from the local market. The barks of *Azadirachta indica* andMilletiapinnatawerecollectedfromthelocalareaofPerambalur.Barkswerethoroughlywashed to remove dirt residues and oven dried overnight at 70 °C. Bark powder (20 g) was extracted in 200 ml 95% ethanol for 4-5 h in a Soxhlet extractor (4-6 cycles) until the solvent in the siphon tube became colorless. The extract was filtered and evaporated to dryness in vacuum on a rotary evaporator.

Qualitative phytochemical analysis

Test for tannins: 10 ml bromine water was added to 0.5 g aqueous extract. Decolorization of bromine water showed the presence of tannins.

Test for saponins: 5.0 ml of distilled water was mixed with aqueous crude plant extract in a test tube and mixed vigorously. The foam was mixed with a few drops of olive oil and mixed vigorously and the foam appearance indicated the presence of saponins.

Tests for flavonoids: test with alkaline reagents. 2 ml of 2.0% NaOH mixture was mixed with aqueous plant crude extract; A concentrated yellow color was produced which became colorless when 2 drops of dilute acid were added to the mixture. This result indicated the presence of flavonoids.

Detection of phenols: Iron chloride test: Extracts were treated with a few drops of 10% iron chloride solution. The formation of a blue-black color indicates the presence of phenol.

Test for steroids: To the 5 ml of crude aqueous plant extract was added 2 ml of chloroform andconcentratedH2SO4.Aredcolorappearedinthelowerchloroformlayer,indicatingthepresenceof steroids.

Test for quinones:

About 0.5 g of plant extract were taken and 1 ml of extract and 1 ml of concentrated H_2SO_4wereadded. The formation of a red color indicates the presence of quinones.

Test for carboxylic acid:

One ml of the different extracts were treated separately with a few ml of sodium bicarbonate solution. Bubbling (due to the release of carbon dioxide) indicates the presence of carboxylicacid.

Detection of alkaloids

Mayer test: extracts were individually dissolved in dilute hydrochloric acid and filtered. were used to test for the presence of alkaloids. The filtrates were treated with Mayer's reagent. The formation of a yellow cream colored precipitate indicates the presence of alkaloids.

Test for coumarins:

0.5 ml of the moistened extracts were placed in a test tube. The mouth of the tube was covered with filter paper treated with 1N NaOH solution. The test tube was placed in boiling water for a few minutes and then the filter paper was removed and examined under UV light for yellow fluorescence indicating the presence of coumarins.

Tests for lignins:

Labat Test: When gallic acid is added to the test sample, it results in the formation of an olive green color that reads positive

Thin Layer Chromatography

Thin layer chromatography has been used an as analytical tool, especially in organic chemistry, due to its high separation speed and its applicability to a large number of chemical compounds. The high sensitivity

of the TLC is used to check the purity of the samples. With the help of DC, it can be seen whether a reaction is complete and has taken the expected course. The homogeneity of the compounds was monitored by these TLC plates and visualized by iodine vapor.
Separation: 10 µl of sample are applied to prepared TLC plates (MREK), activated at 100 º C for5 min and then placed in a mobile phase beaker. A glass chromatography tank saturated with the mobile phase for 30 min was used for the linearly increasing development. The developed plates were air dried and then UV scanned at 275nm.

Lipase Inhibition Test

Lipase inhibitory activity of bark extracts performed according to the method described by Kim et al. with some modifications. Reaction volume of 2000 µl, containing 100 µl bacterial lipase, previously isolated stock enzyme and 200 µl of different concentrations of bark extracts (test concentrations: 500, 100, 150 and 200 µg/ml) in 1 ml 0.1 M Tris-HCl buffer 100 µL mM CaCl2,pH 7.0, were pre incubated at 37°C for 15 min. The reaction was started by adding 5 µl of 10 mMp-NPB in dimethylformamide and allowed to proceed at 37°C for 30 min. The lipase inhibitory activity of bark extracts was determined by measuring the hydrolysis of p-NPB to p-nitrophenolat 405 nm. Inhibition of lipase activity was expressed as a percent decrease in optical density when pancreatic lipase was incubated with cortical extracts. Atorvastatin was used as a positive control(test concentration: 50–200 µg/ml,).
(A-a)-(B-b)/(A-a)
where A is the activity without inhibitor, a is the negative control without inhibitor, B is the activity with inhibitor, and b is the negative control with inhibitor.

Cholesterol Esterase Inhibition Assay

Cholesterol esterase inhibitory activity of bark extracts was performed using pancreatic lipase with minor modifications. Reaction volumes of 200 µl containing various concentrations of bark extracts (test concentrations: 50-200 µg/ml) were preincubated with 50 µl of 24 mM taurocholic acid, 5 µl of 8 mM p-NPB in acetonitrile in 0.1 M sodium phosphate buffer, 0.1 M NaCl, pH 7.0at 25°C for 10 min The reaction was started by adding 42.5 µl (1.25 µg/ml) cholesterol esterase enzyme and the change in absorbance was monitored at 405 nm at 25°C

In Vitro Hemolytic Assay

The hemolytic effect of various extracts on human and rat erythrocytes was evaluated using washed erythrocytes (RBCs). Human blood is collected for the preparation of RBC. The blood is diluted with PBS (pH7.4) and a 2% erythrocyte suspension is prepared. Human erythrocytes were obtained from the peripheral blood (O-positive) of a healthy volunteer. The 100 µl of blood was mixed with ONE volume of sterile 0.85% NaCl saline. And a concentration of 100µgextractisadded and incubated for 30 minutes. SDS is used as a positive control.PBS was used as a negative control. Percent hemolysis recorded at 550 nm
OD of test-OD of NC /OD of SDS treated x100

RESULT AND DISCUSSION

The results of the phytochemical analysis of the leaf extracts in various solvents has shown a remarkable variation in the presence the above studied phytochemical compounds in the studied taxa. The detailed investigations of phytochemicals in various solvents are shown in TABLE 1. Test for Tannin and Test for Lignin were negative. Phytochemical analysis of the solvent extracts of the bark revealed the presence of flavonoids, tannins, phenol, saponins, alkaloids, coumarins which was presented uniquely in root TLC of bark extract with Rf value were recorded(plate 3). In the extracts of three plant parts, 7 different Rf values appeared in the range of 0.2to 0.8(table 2). Positive test of alkaloids are orange spots; and positive test of terpenoids and steroids are violet, blue, grey or green spots were noted.The presence of alkaloids, terpenoids and steroids conform with the previous works.

Millettia pinnata *Azadirachta indica* *Cinnamomum verum*

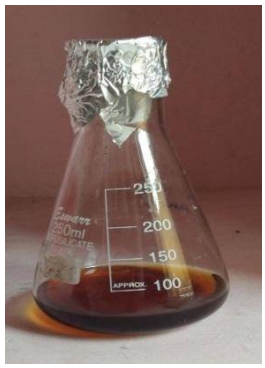

Fig.1.Bark samples used for extraction

Table:1Phytochemical analysis

TEST	RESULT
Test for Flavonoids	Positive
Phenolic compounds	Positive
Test for Sterols	Positive
Test for Quinones	Positive
Test for Carboxylgroup	Positive
Test forTannin	Negative
Test for Saponin	Positive
Test for Coumarin	Positive
Test for Lignin	Negative

Table:2TLC-Visible in UV

S.No	DISTANCE	Rf VALUE
1	1.5	0.2
2	2	0.26
3	2.5	0.33
4	3	0.4
5	5	0.66
6	5.5	0.73
7	6	0.8

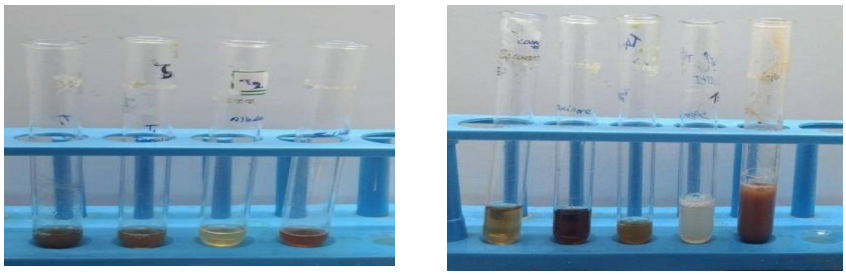

Fig.2 Phytochemical Test

Fig.3TLC separation of compound

Antilipase and esterase Activity

Bark extracts of the tested formulation showed a moderate and dose-dependent antilipase activity. Compared to the standard drug (IC50 26.78 ± 2.45 µg/ml), both extracts showed moderate activity.The dose-response relationship of bark extracts for antilipase activity is given in Table 3. The percentage of inhibition of lipase activity was 28, 36, 59 and 69% at 25, 50, 75and 100 µg and 17, 22, 35, 52% as standard. The cholesterol esterase inhibitory activity of bark extracts is given in Table 5. Ethanol barks extract had significantly high activity compared to the standard. The IC50 values for lipase inhibition were 64 µg for bark extract and 103µgforstandard. The IC50 values of esterase inhibition among the extracts are 123 µg and the standard value is 158 µg (Figure 5). Biocompatibility shows that the extract contains 1.2% hemolytic, indicating safe use and non-toxic nature(Table 5).

Table5. Percentage of Lipase and esterase inhibition of bark extracts

Conc. of extract µg/mL	Lipase inhibition	Esterase
25	28	14
50	36	18
75	59	34
100	69	66
	64	123
STANDARD		
25	17	12
50	22	18
75	35	22
100	52	60
	103	158

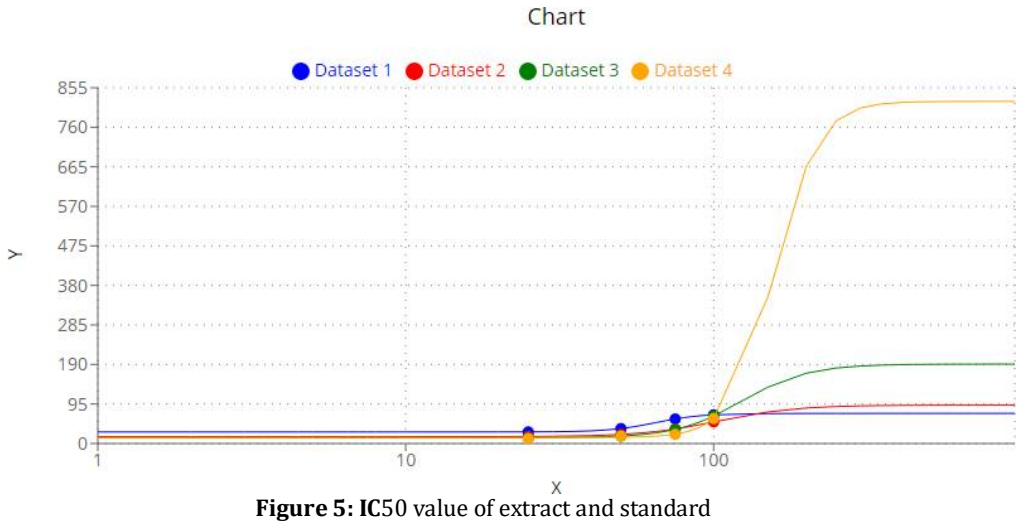

Figure 5: IC50 value of extract and standard

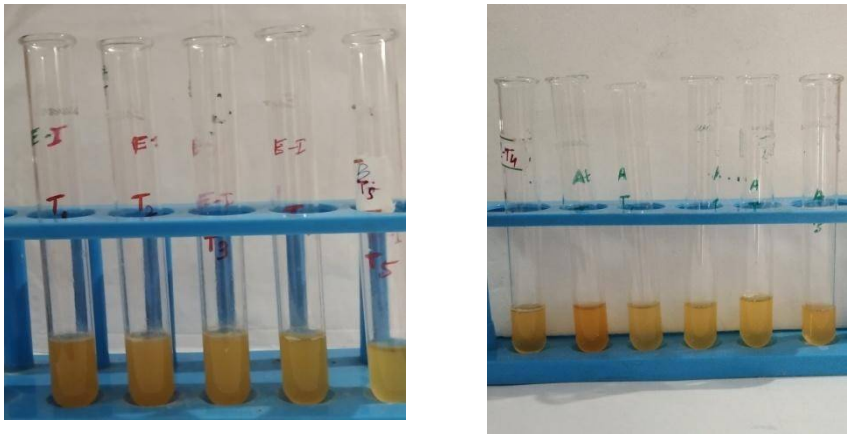

Fig.4.Esterase and lipase inhibition assay

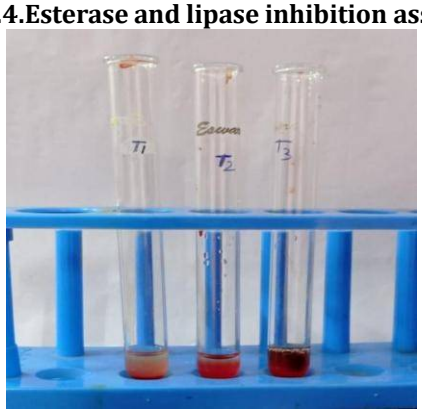

Fig.5.Hemolytic test

CONCLUSION

The goal of this study is to look for bioactive components in ethanolic extracts of *Azadirachta indica* and *Millettia pinnata* bark, as well as the extract inhibited lipase and esterase at varying concentrations further supports the usefulness of the extract in decreasing cholesterol. This could lead to the development of novel medications that target Synthase of squalene

ACKNOWLEDGEMENT

The authors acknowledgement Chancellor Shri.A.Srinivasan , Dhanalakshmi Srinivasan College of Institutions for the Financial support of this work.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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CITATION OF THIS ARTICLE

Subasankari Kumaresan, RabiyaS, Sujithra M, S.Sathish and Narendra Kumar Singh: Anti-Lipidemic Activity of Bark Extract-A Polyherbal Extraction and Phytochemistry Analysis. Bull. Env.Pharmacol. Life Sci., Spl Issue [5]: 2022: 544-550.