EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 8
Issue: 12
227-232
Year: 2021

EVALUATION OF PHYTOCHEMICALS OF *ANOGEISSUS ACUMINATA* LEAVES

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Article Received on 29/09/2021 **Article Revised on 19/10/2021** **Article Accepted on 09/11/2021**

ABSTRACT
Plants are the "sleeping giant of drugs development". They forms a reservoir of potential useful untapped sources of drugs that have been serving mankind since dawn of civilization. Hence it is necessary to explore the possibility of using the traditional medicines with proper chemical and pharmacological profiles. *Anogeissus acuminata* belongs to *combretaceae* family and is a wild evergreen tree of variable size, found both wild and cultivated throughout India. *Anogeissus acuminata* is known to be a rich source of carbohydrates, fat, protein, aminoacids, anthocyanins, leucoanthocyanins, saponins and alkaloids. In the present study the leaves of *Anogeissus accuminata*, commonly known as Button Tree were selected for preliminary phytochemical investigation. The dried leaves were subjected to successive solvent extraction with the solvent in increasing order of polarity, viz. petroleum ether, chloroform, alcohol and aqueous (water) using maceration process. All the extracts were subjected to qualitative chemical tests and carbohydrate, steroids, alkaloids and flavonoids were found to be present.

KEYWORDS: Phytochemical, Traditional medicine, *Anogeissus Acuminate* Leaves.

INTRODUCTION
It is a large deciduous tree. Leaves elliptic or laceolate, entire, acute or mucronate, glabrous above, appressed pubescent beneath. Flowers are greenish. Fruits are brown, wings denate often lobed.[1]

Anogeissus acuminata is a moderate size tree with small leaves, which falls earlier on the dry season. Before falling the foliage of these turns a beautifully yellowish red, growing in South Africa and Arabian Peninsula. Leaves of the plant are used in traditional and tribal medicine of Andhra Pradesh to treat various conditions.

Shrubs to small trees, 2-20 m high; bark grey, whitish or dark brown; branchlets blackish, brown or coppery, 1-7 mm thick, terete, minutely grey-pubescent to glabrous. Leaves opposite to sub opposite, 3-8 x 1.5-3 cm, elliptic to oblong-elliptic or ovate to sub orbicular, acute, obtuse or mucronate or sometimes shortly acuminate at apex, base acute to obtuse or subcuneate and sometimes decurrent, thinly coriaceous, sparsely adpressed greyish-pilose to glabrous above, sparsely to densely adpressed tomentellous or glabrous beneath; lateral nerves 4-8 pairs, obscure to faint above; intercostae inconspicuous above, faint beneath, scalariform to reticulate; petioles 1-8 mm long. Inflorescence: heads 1-2 cm across, axillary and terminal, solitary or occasionally in pairs; peduncles 0.5- 2 cm long; bracts on peduncles 1-2 pairs, subfoliaceous, ovate to lanceolate or oblanceolate, 3-10 mm long, sub persistent; bracteoles spathulate or ovate-elliptic, 1-2 mm long. Calyx tube 2-4 mm long, tawny-or ferrugineous-tomentellous; expanded portion cupular or shortly campanulate, 1.5-2 x 2-2.5 mm, glabrous or pubescent only at base; teeth 5-6, triangular, ca 0.5 mm long. Petals 0. Stamens 2-4 mm long; anthers oblong to suborbicular, ca. 0.5 mm long. Ovary 1-celled, ovules 2, style 2-4 mm long. Fruits 3-7 x 5-8 mm (excluding beak), puberulous to glabrous, reddish-brown, often glossy; wings 2, membranous, entire or slightly undulate along margins; beak 1.5-4 mm long; seeds ovoid, 3-5 x 2.5-3 mm.[2]

Fig. 1: Anogeissus acuminate leaf.

The timber of this plant has potential value because of its strength and working qualities. The wood apart from being one of the toughest is also ideal for making handles of tools, rafters, bobbins, wheel spokes and furniture. Plants of A. acuminata are found in patches scattered throughout the arid, semi-arid areas. It has been reported that A. acuminata has valuable medicinal properties.

Chemical constituent

Anogeiussus acuminata is known to be a rich source of carbohydrates, fat, protein, aminoacids, anthocyanins, leucoanthocyanins, saponins and alkaloids.

The ground seeds on extraction with petroleum ether gave 33 percent of bright yellowish oil which contains oleic and linoleic. The seed also contains myristic, palmitic acid, β-sitosterol, stigmasterol, jujubosides A and B (saponins), spinosin and its derivatives.[3]

Analyses of the leaves from four different states, Uttar pradesh, Gujarat, West Bengal and orissa gave the following range of values crude protein 12.9-16.9, ether ext (fat), 1.5-2.7, total ash 10.2-11.7, calcium 1.42-3.59, phosphorus 0.21-0.33%. The digestible nutrients present in the leaves from Izatnagar were as follows crude protein 3.1, ether extract 1.1, carbohydrates 25.1, and total digestible nutrients 30.7%. The mineral constituents present in samples of leaves from Uttar pradesh are calcium 2.81-3.74, phosphorus 0.17-0.27, magnesium 0.46-0.83, potassium 0.47-1.57, and sulphur 0.13-0.33%. Ceryl alcohol and two alkaloids protopine and berberine have been isolated from the leaves.[4]

MATERIALS AND METHODS

Preparation of Extracts of *Anogeissus Acuminata*

The leaves of *Anogeissus acuminate* were collected from TIRUPATI district, Andhra Pradesh, India and authenticated by Dr. K. MADHAVA CHETTY, Assistant Professor, Department of BOTONY, SRI VENKATESWARA UNIVERSITY, TIRUPATI. - 517502 A. P. INDIA. The shade dried leaves were cut into small pieces and were powdered mechanically to obtain a coarse powder. The coarse powder was then stored in a clean, dry and airtight container.[5]

The powdered material was subjected to maceration. The solvents used were petroleum ether (60-80º), Chloroform, methanol and distilled water. The powdered material of *Anogeissus acuminata* was evenly packed in a conical flask (2 liter capacity) for extraction for about seven days with different solvents. Then the macerated product was filtered using muslin cloth and resultant product was kept in water bath to get the final product.[6]

All extract viz., petroleum ether, methanol, chloroform, and aqueous extract were first subject for the preliminary phytochemical investigation.

PHYTOCHEMICAL INVESTIGATION[7-12]

Tests for Carbohydrates

The test solution was prepared by dissolving test extract with water, hydrolyzed with 2N hydrochloric acid and subjected to following tests.

Molish's test (General test): To the test solution, added few drops of α-naphthol solution in alcohol, shaken and added concentrated H_2SO_4 from sides of the test tube was observed for violet ring at the junction of two liquids.

For Reducing Sugars

Fehling's test: 1 ml Fehling's A and 1ml Fehling's B solutions was mixed and boiled for one minute. Added equal volume of test solution. Heated in boiling water bath for 5-10 min was observed for a yellow, then brick red precipitate.

Benedict's test*:* Equal volume of Benedict's reagent and test solution in test tube were mixed. Heated in boiling water bath for 5 min. Solution may appear green, yellow or red depending on amount of reducing sugar present in test solution.

Tests for Monosaccharides

Barfoed's test: Equal volume of Barfoed's reagent and test solution were added. Heated for 1-2 min, in boiling water bath and cooled. Observed for red precipitate.

Tests for Hexose Sugars

Cobalt-chloride test*:* 3 ml of test solution was mixed with 2ml cobalt chloride, boiled and cooled. Added $FeCl_3$ drops on NaOH solution. Solution observed for greenish blue (glucose), purplish (Fructose) or upper layer greenish blue and lower layer purplish (Mixture of glucose and fructose).

Tests for Non-Reducing Sugars

Test solution does not give response to Fehling's and Benedict's test.

Test for Non-Reducing Polysaccharides (Starch)

Iodine test*:* mix 3 ml test solution and few drops of dilute Iodine solution. Blue color appears; it disappears on boiling and reappears on cooling.

Tannic acid test for starch*:* With 20% tannic acid, test solution was observed for precipitate.

Tests for Proteins

The test solution is prepared by dissolving the extract in water.

Biuret test (General test): To 3 ml T.S added 4% NaOH and few drops of 1% C_uSO_4 solution observed for violet or pink color.

Million's test (for proteins)*:* Mixed 3 ml T.S. with 5 ml Million's reagent, white precipitate. Precipitate warmed turns brick red or precipitate dissolves giving red colour was observed.

Xanthoprotein test (For protein containing tyrosine or tryptophan): Mixed 3ml T.S. with 1 ml concentrated H_2SO_4 observed for white precipitate.

Test for protein containing sulphur

Mixed 5 ml T.S. with 2 ml 40% NaOH and 2 drops 10% lead acetate solution. Solution was boiled it turned black or brownish due to Pbs formation was observed.

Tests for amino acids

Ninhydrin test (General test): 3 ml T.S. and 3 drops 5% Ninhydrin solution were heated in boiling water bath for 10 min. Purple or bluish colour observed.

Test for Tyrosine*:* Heated 3 ml T.S. and 3 drops Million's reagent. Solution observed for dark red colour.

Test for Tryptophan**:** To 3 ml T.S. added few drops glycoxalic acid and concentrated H_2SO_4 observed for reddish violet ring at junction of the two layers.

Test for Cysteine: To 5 ml. T.S. add few drops of 40% NaOH and 10% lead acetate solution. Boil. Black ppt. of lead sulphate is formed.

Tests for Steroid

Salkowski Reaction: to 2 ml of extract, 2 ml chloroform and 2 ml concentrated H_2SO_4 was added. Shaked well, whether chloroform layer appeared red and acid layer showed greenish yellow fluorescence was observed.

Liebermann-Burchard Reaction*:* Mixed 2ml extract with chloroform. Added 1-2 ml acetic anhydride and 2 drops concentration H_2SO_4 from the side of test tube observed for first red, the blue and finally green colour.

Libermann's reaction: Mixed 3 ml extract with 3 ml acetic anhydride. Heated and cooled. Added few drops concentrated H_2SO_4 observed for blue colour.

Tests for Flavonoids

The flavonoids are all structurally derived from the parent substance called flavone. The flavonoids, which occur in the form of free, as well as, bound sugars called glycosides. For this reason, when analysing flavonoids, it is usually better to examine the flavonoids in hydrolysed plant extracts.

Preparation of test solution: To small amount of extract equal volume of 2M hydrochloric acid is added and heated the test tube for 30-40 min at 100^OC, allowed to cool, filtered and extracted with ethyl acetate. The ethyl acetate extract was concentrated to dryness, followed the test for flavonoids to ethyl acetate fraction by dissolving the residue with ethyl acetate.

Shinoda test: Test solution with few fragments of magnesium ribbon and conc. hydrochloric acid shows pink to magenta red colour.

Zn/Hcl reducing test: Test solution with zinc dust and few drops of hydrochloric acid shows magenta red colour.

Tests for Glycosides

The test solution was prepared by dissolving extract in alcohol 90% or aqueous alcoholic solution.

Tests for Cardiac Glycosides

Baljet's test*:* A test solution observed for yellow to orange colour with sodium picrate.

Legal's test (For cardenoloids)*:* To aqueous or alcoholic test solution, added 1 ml pyridine and 1 ml sodium nitroprusside observed for pink to red colour.

Test for deoxysugars (Kellar Killani test): To 2 ml extract added glacial acetic acid, one drop of 5% $FeCl_3$ and concentrated H_2SO_4 observed for reddish brown colour at junction of the two liquid and upper layers bluish green.

Libermann's test (For bufadenolids): Mixed 3 ml extract with 3 ml acetic anhydride. Heated and cooled. Added few drops concentrated H_2SO_4 observed for blue colour.

Tests for Saponin Glycosides

Foam test: The drug extract or dry powder was shaked vigorously with water. Persistent foam was observed.

Haemolytic test: Added test solution to one drop of blood placed on glass slide. Haemolytic zone whether appeared was observed.

Tests for Antraquinone Glycosides

Brontager's test: To 3 ml. extract, add dil. H2SO4. Boil and filter. To cold filtrate, add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammoniacal layer turns pink or red.

Modified Brontager's test: To 5 ml extract, add 5 ml. 5% $FeCl_3$ and 5 ml. dil. HCl. Heat for 5 min. in boiling water bath. Cool and add benzene or any organic solvent. Shake well. Separate organic layer. Add equal volume dilute Ammonia. Ammoniacal layer shows pinkish red colour.

Tests for Alkaloids

The test solution was prepared by dissolving extracts in dilute hydrochloric acid.

Dragendroff's test: To 2-3 ml filtrate added few drops Dragendroff's reagent observed for orange brown precipitate.

Mayer's test: 2-3 ml filtrate with few drops Mayer's reagent observed for cream coloured precipitate.

Hager's test: 2-3 ml filtrate with Hagers reagent observed for yellow precipitate.

Wagner's test: 2-3 ml filtrate with few drops of Wagner's reagent observed reddish brown precipitate.

Tests for Tannins and Phenolic Compounds

To 2-3 ml test solution, added few drops of whether showed following was observed:

5% $FeCl_3$ solution: - Deep blue-black coloured.
Lead acetate solution: - White precipitate.
Gelatin solution: - White precipitate.
Bromine water: - Decoloration of bromine water.

Tests for Vitamins

Test for Vitamin A: Dissolve a quantity equivalent to 10-15 units in 1 ml of chloroform and add 5 ml of antimony trichloride solution, a transient blue colour is produced immediately.

Test for Vitamin C: Dilute 1 ml of 2% w/v solution with 5 ml of water and add 1 drop of freshly prepared 5% w/v solution of sodium nitroprusside and 2 ml of dil. Sodium hydroxide solution. Add 0.6 ml of HCL drop wise and stir, the yellow colour turns blue.

Test for Vitamin D: Dissolve a quantity equivalent to about 1000 units of vitamin D activity in chloroform and add 10 ml of antimony trichloride solution, a pinkish-red colour appears at once.

RESULTS

Qualitative chemical tests were conducted for all the extracts of leaves of *Anogeissus acuminata* to identify the various phytoconstituents. The various tests and reagents used are given below and observations are recorded in the Table.

Table 2: Identification of the various phytoconstituents of *Anogeissus acuminate.*

Chemical Tests	Extracts			
	Petroleum ether	Chloroform	Alcohol	Aqueous
Test for Carbohydrates:				
Molish's test (General test)	+	+	+	+
a) Test for reducing sugars				
1) Fehling's test	+	+	+	+
2) Benedict's test	+	+	+	+
b) Test for Monosaccharides				
1) Barfoeds test	+	+	+	+
c) Test for Hexose Sugars				
1) Cobalt chloride test	-	-	-	+
d) Test for Non-reducing sugars	-	-	-	-
e) Test for non-reducing polysaccharides (starch)				
1) Iodine test	-	-	+	+
2) Tannic acid test for starch	-	-	+	+
Test for Proteins:				
a) Biuret test (General test)	-	-	-	+

b) Millions test for proteins	-	-	+	+
c) Xanthoprotein test	-	-	-	-
d) Test for proteins containing sulphur	-	-	-	-
Test for Amino acids:				
a) Ninhydrin test (General test)	-	-	-	-
b) Test for tyrosine	-	-	-	-
c) Test for tryptophan	-	-	-	-
d) Test for cysteine	-	-	-	-
Test for Steroids:				
a) Salkowski reaction	-	-	-	-
b) Liebermann – Burchard reaction	-	-	-	-
c) Liebermann reaction	-	-	-	-
Test for Flavanoids:				
Shinoda test	-	-	+	+
Zn/Hcl reducing test	-	-	+	+

Table 3: Preliminary Phytochemical Screening of *Anogeissus acuminate.*

Chemical Tests	**Extracts**			
	Petroleum ether	**Chloroform**	**Alcohol**	**Aqueous**
Test for Carbohydrates:				
Molish's test (General test)	+	+	+	+
a) Test for reducing sugars				
1) Fehling's test	+	+	+	+
2) Benedict's test	+	+	+	+
b) Test for Monosaccharides				
1) Barfoeds test	+	+	+	+
c) Test for Hexose Sugars				
1) Cobalt chloride test	-	-	-	+
d) Test for Non-reducing sugars	-	-	-	-
e) Test for non-reducing polysaccharides (starch)				
1) Iodine test	-	-	+	+
2) Tannic acid test for starch	-	-	+	+
Test for Proteins:				
a) Biuret test (General test)	-	-	-	+
b) Millions test for proteins	-	-	+	+
c) Xanthoprotein test	-	-	-	-
d) Test for proteins containing sulphur	-	-	-	-
Test for Amino acids:				
a) Ninhydrin test (General test)	-	-	-	-
b) Test for tyrosine	-	-	-	-
c) Test for tryptophan	-	-	-	-
d) Test for cysteine	-	-	-	-
Test for Steroids:				
a) Salkowski reaction	-	-	-	-
b) Liebermann – Burchard reaction	-	-	-	-
c) Liebermann reaction	-	-	-	-
Test for Flavanoids:				
Shinoda test	-	-	+	+
Zn/Hcl reducing test	-	-	+	+

DISCUSSION

Anogeissus acuminata belongs to *combretaceae* family and is a wild evergreen tree of variable size, found both wild and cultivated throughout India. It is a large deciduous tree. Leaves are eulliptic or laceolate, entire, acute or mucronate, glabrous above, appressed pubescent beneath. Flowers are greenish. Fruits are brown, wings denate often lobed.

Anogeissus acuminate is a moderate size tree with small leaves, which falls earlier on the dry season. Before falling the foliage of these turns a beautifully yellowish red, growing in South Africa and Arabian Peninsula. Leaves of the plant are used in traditional and tribal medicine of Andhra Pradesh to treat various conditions.

Anogeissus acuminata is known to be a rich source of carbohydrates, fat, protein, aminoacids, anthocyanins, leucoanthocyanins, saponins and alkaloids. The ground seeds on extraction with petroleum ether gave 33 percent of bright yellowish oil which contains oleic and linoleic. The seed also contains myristic, palmitic acid, β-sitosterol, stigmasterol, jujubosides A and B (saponins), spinosin and its derivatives.

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Reports suggest that flavonoid containing herbal drugs had an effect on collagen fibres pre-treated with acrylic polymer followed by treatment of tannin containing herb exhibited an increase in hydrothermal stability, which reveals that tannin containing herbs stabilizes Type-1 collagen which is unique connective tissue protein which promote burn wound healing.

Flavonoids and tannin containing compounds also help in rapid cleaning of purulent wounds and shortening of duration of treatment.

CONCLUSION

Anogeissus acuminata is rich source of carbohydrates, fat, protein, aminoacids, anthocyanins, leucoanthocyanins, saponins and alkaloids. The ground seeds on extraction with petroleum ether gave 33 percent of bright yellowish oil which contains oleic and linoleic. The seed also contains myristic, palmitic acid, β-sitosterol, stigmasterol, jujubosides A and B (saponins), spinosin and its derivatives.

The relationship between man & plants have been close throughout the development of human culture with the disease in the understanding of human disease there has been continued interest in the drugs from the plant kingdom.

Nature's beauty in terms of health care is appreciated by developing various systems of traditional system (Ethnomedicine). There is growing focus on the importance of medicinal plants in the traditional health care system in solving health care problems. Because of this awareness plant material & herbal remedies derived from them represents substantial portion of the global market.

Acknowledgements

We thank the management of JJT University for providing necessary facilities to carry out our research work. We also acknowledge Rakesh Kumar Jat, for his valued suggestion.

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