PRELIMINARY PHYTOCHEMICAL SCREENING OF VARIOUS SOLVENT EXTRACTS OF FICUS VASTA FORSSK. (FAMILY: MORACEAE), AN ETHIOPIAN FIG PLANT

Samuel Tadesse¹, Kumar Ganesan², Suresh Kumar P. Nair², Neethu Letha³ and Sharmila Banu Gani⁴*

¹Department of Physiology, College of Public Health and Medical Sciences, Jimma University, Jimma 378, Ethiopia.
²Department of Biochemistry, College of Public Health and Medical Sciences, Jimma University, Jimma 378, Ethiopia.
³Department of Zoology, Government Women’s College, Trivandrum, Kerala, India.
⁴Department of Zoology, NKR Government Arts College for Women, Namakkal-637001, Tamilnadu, India.

ABSTRACT

Background: Ficus vasta Forssk. (Amharic: warka) is found in Ethiopia and Yemen, popularly known as a fig plant. Plants of genus Ficus belongs to family Moraceae and have been extensively used for controlling the a variety of diseases. Purpose of the study: The aim of the present study was to perform preliminary phytochemical screening of aqueous, methanol, hexane and ethanol extracts of leaves, stems and fruits of Ficus vasta. Methods: The extracts were subjected to qualitative phytochemical screening using standard procedures. Results: The qualitative phytochemical screening of this plant confirmed the presence of various phytochemicals like alkaloids, proteins and aminoacids, Anthraquinones, flavonoids, saponins, steroids, total phenol and tannins. Whereas glycosides and triterpenoids were found to be absent. Conclusion: This preliminary study draws attention to the need for further studies of the active secondary metabolites identified in the reported species for the treatment of many diseases also to understand their mode of action in controlling various dreadful diseases.

KEYWORDS: Ficus vasta Forssk, screening, solvent extracts, secondary metabolites.
INTRODUCTION
Oxidative stress caused by reactive oxygen species (ROS) is associated with the pathogenesis of a numerous dreaded chronic diseases such as diabetes, cancer, atherosclerosis, coronary artery diseases and other degenerative diseases. ROS causes tissue damage includes intracellular protein, lipids and DNA damages and oxidation of membrane bound and mitochondrial enzymes. The use of antioxidants derived from plants such as flavonoids and polyphenols has been most valuable in the anticipation of these dreaded diseases. Phenolic compounds are recognized as radical scavengers to slake oxygen-derived radicals by donating its hydrogen atom and they have revealed to be nullifying free radicals. Many researches have exposed that these antioxidant agents have antihyperglycemic, antiinflammatory, antitumor, anticarcinogenic, antibacterial and antiviral activities. Eating of natural antioxidants has been connected with diminished risks of diabetes, cancer, cardiovascular disease and ageing. Nowadays, it has been a global trend towards the consumption of the natural phytochemical present in herbs, oilseeds, fruits and vegetables.

*Ficus vasta* Forssk. (Amharic: warka) is found in Ethiopia and Yemen, Saudi Arabia and Tanzania popularly known as a fig plant. Plants of genus *Ficus* belongs to family Moraceae, is a huge tree growing more than 20 metre height. In conventional medicine, the plant have been extensively used for analgesic, rheumatism and intestinal worms. The previous study investigated on the fruit extract, employed as antibacterial, cytotoxicity and antihepes and antihelmintic activities. Based upon ethanobotanical survey of Ethiopian indigenous medicinal plants, the plant of *F. vasta* have been selected to prove scientifically having phytoactive compounds. The phytochemicals generated data from the four different extracts of these plants may be used as tools for quality control of drugs in the future, for the healing of a diversity of disease conditions.

MATERIALS AND METHODS
Chemicals
Ferric chloride, HCl, Dragendorff 's reagent, hexane, benzene, carbon tetrachloride, chloroform, H₂SO₄, Folin-Ciocalteu reagent were purchased from Chemico Glass & Scientific Company, Erode, Tamilnadu, India. All the chemicals used in this experiment were of analytical grade.

Collection and authentication of plant material
Ficus vasta Forssk was collected from Jimma University Garden, Jimma, South West Ethiopia in the month of October-2014. The plant has been taxonomically identified and authenticated by the Jimma University Botanist Dr. Ramesh Moochikal and kept in Jimma University Botanical Science and Herbarium for future references.

**Methanol extract of leaves, stem and fruits of Ficus vasta Forssk**

The shadow dehydrated roughly powdered of leaves and stem of F. vasta was engrossed and haul out with methanol for 72hrs. After finishing point, the defatted solutions were sieved by filter paper Whatmann No.1 to eliminate any contamination. The extract was intensified by vaccum dessicator to reduce the degree; the concentrated samples were relocated to another beaker and the residual solvent was further vaporized. Finally the dark greenish yellow coloured extract was formed and again it was kept in a vaccum dessicator to get rid of unnecessary wetness. Dehydrated extract was stored in sealed a container and which was used for qualitative phytochemical screening.

**Aqueous, ethanol and hexane extracts of leaves, stem and fruits of Ficus vasta Forssk**

The residues left after methanol extraction was dehydrated and then engrossed separately with aqueous and hexane respectively up to 3days. After finishing point of extraction, the organic solvents were eliminated by vaccum dessicator. Dark greenish yellow colour extracts were formed and then stored in a sealed container for further studies.

**Preliminary phytochemical studies**\(^{[12, 13]}\)

The extracts obtained (benzene, ethanol, carbon tetrachloride, and aqueous) were employed to the subsequent phytochemical screening.

**Test for Alkaloids**

a) **Dragendorff’s test**

Take 1ml of the solvent extract, add equal volume of distilled water followed by 1ml of 2molar solution of HCl added until acidification reaction takeplace. To Add this 1ml of Dragendorff’s reagent. Orange or red colour is formed, indicated that the occurrence of alkaloids.

b) **Hagger’s Test**

Take 1ml of the solvent extract in a cleaned test tube, add 1ml of Hager’s reagent. Yellow precipitate is formed, indicated that the occurrence of alkaloids.

c) **Wagners Test**
Take 1ml of solvent extract acidified with 1ml of 1.5 % v/v of HCl and add 1ml of wagners reagent. Formation of yellow or brown precipitate, which indicated that the occurrence of alkaloids.

d) Mayers Test
Take 1ml of Mayers reagent, add 1ml of solvent extract. White or pale yellow precipitate is formed, indicated that the occurrence of alkaloids.

Test for Carbohydrates

a) Anthrone Test
Take 1ml of solvent extract and 10ml of distilled water in a test tube, shaken vigorously and filtered. To this filtrate, add 1ml of anthrone reagent and mixed. Green or blue color is formed, indicated that the occurrence of carbohydrates.

b) Benedict's Test
Take 1ml of solvent extract and 10ml of water in a test tube, shaken vigourously and filtered. To this filtrate, add 3ml of Benedict's reagent and kept in a boiling water bath for 5min. Development of red colour indicated that the occurrence of reducing sugar.

c) Fehlings Test
Take 1ml of solvent extract and 10ml of water in a test tube, shaken vigourously and filtered. To this filtrate, add 1ml of Fehlings solution A and 1ml of Fehlings solution B and kept in a boiling water bath for 5min. Development of red colour indicated that the occurrence of reducing sugar.

d) Molisch's Test
Take 1ml of solvent extract and 10ml of water in a test tube, shaken vigourously and filtered. To this filtrate, 1ml of Molisch reagent were added followed by few drops of Conc. H$_2$SO$_4$ added in the side of the test tube. Formation of two junction, which indicates the occurrence of carbohydrates.

Test for flavonoids

a) Shinods test
Take 1ml of solvent extract diluted with 3ml of ethanol followed by 2ml dilute HCl and pinch of Mg in a test tube, shaken gently. Appearance of pink or brown precipitate indicated that the occurrence of flavonoids.

b) With Conc. H$_2$SO$_4$ test
when treated with Con. $\text{H}_2\text{SO}_4$, appearance of the following colour like yellow colour (anthocyanins), yellow colour change to orange (flavones); orange colour change to crimson (flavonones) respectively.

**Test for Glycosides**

**Molisch Test**

Take 1ml of solvent extract and 10ml of water in a test tube, shaken vigourously and filtered. To this filtrate, 1ml of Molisch reagent were added followed by few drops of Conc. $\text{H}_2\text{SO}_4$ added in the side of the test tube. Formation of two junction, which indicates the occurrence of glycosides.

**Test for proteins and free amino acids**

1. **Millions test**

Take 1ml of solvent extract with 1ml of Millions reagent, shake gently. Appearance of cherry red color indicated that the occurrence of free amino acid.

2. **Ninhydrin test**

Take 1ml of solvent extract with 1ml of Ninhydrin reagent, shake gently Formation of violet color indicated that the occurrence of free amino acids.

3. **Biuret test**

Take 1ml of solvent extract with 1ml of 10% NaOH and 1ml of 1% copper sulphate in a test tube, shake gently. Development of purple color indicated that the occurrence of proteins.

**Test for gums and mucilage**

**With 95% alcohol**

Take 1ml of solvent extract with 25 ml of 95% alcohol in a test tube, shake gently and filtered. The residue was air dried and examined for its bulging property. It indicated that the occurrence of gums and mucilages.

**Test for anthraquinones**

Take 2ml of the solvent extracts acid hydrolysed with Conc. $\text{H}_2\text{SO}_4$ followed by extracted with benzene. Add 2ml of dilute ammonia. Appearance of rose pink color indicated that the occurrence of anthraquinones.

**Test for Saponins**
Foam test
Take 5ml of solvent extracts in a test tubes add a drop of sodium bicarbonate, shaken vigorously and kept it stand for 3min. Development of cloudy white precipitate indicated that the occurrence of saponins.

Test for Sterols
a) Liebermann-Buchards test
Take 1ml of solvent extract in a test tube and add acetic anhydride and kept in a boiling water bath for 5min, then cooled followed by 1ml of Con. H₂SO₄ added along the sides of the test tube. Appearance of green color indicated that the occurrence of steroids.

b) Salkowski reaction
Add 1ml of solvent extract diluted with chloroform and followed by 1ml of Con. H₂SO₄ added along the sides of the test tube. Appearance of two junction/layer indicated that the occurrence of steroids.

Test for fixed oils
Spot test
Take 0.5ml of solvent extract and pressed in between the two filter papers. Formation of oil stains on the paper indicated the existence of fixed oil.

Add 1ml of 0.5N alcoholic KOH and 1ml of solvent extract along with a single drop of phenolphthalein in a test tube. The residues were kept in a boiling water bath for 20min. Appearance of soap or incomplete neutralization of alkali indicated that the occurrence of fixed oils.

Test for triterpenoids
Add 2ml of solvent extract and 1 ml of CHCl₃ followed by 1 ml of acetic anhydride in a test tube and shake gently. Add 1ml of Con. H₂SO₄ added along the sides of the test tube. Appearance of two junction/layer indicated that the occurrence of triterpenoids.

Test for phenolic compounds and tannins
About 5ml of solvent extracts and equal volume of water added and perform the following reagent for confirmation of phenolic compounds and tannins.

Ferric chloride reagents
It gives a violet color

**Gelatin containing sodium chloride**

It gives a white precipitate.

**Lead acetate solution**

It gives a white precipitate.

RESULTS AND DISCUSSION

In the study, preliminary phytochemical investigation has been done in the four extracts (aqueous, methanol, hexane and ethanol) of leaves, stem and fruits of *F. vasta* showed the presence of phytochemical constituents namely alkaloids, proteins and amino acids, Anthraquinones, flavonoids, saponins, steroids, total phenol and tannins. Whereas glycosides and triterpenoids were found to be absent (Table 1).

The preliminary phytochemical analysis investigation on *F. vasta* extract revealed the presence of alkaloids, saponins, flavonoids, polyphenol and tannins, and anthraquinones. Tannins are recognized to be helpful in the treatment of chronic inflammation in tissues and they have notable activity on anticancer.\(^{[14]}\) Thus, *F. vasta* containing these chemical compounds may provide as active principle in the treatment of various cancer.

Flavonoids are phenolic compounds that are acting as principal antioxidants or free radical scavengers and serve as health promoting compound as a results of its anion radicals.\(^{[14]}\) Since these phenolic compounds were originated to be present in the extracts, it might be accountable for the potent antioxidant capacity of *F. vasta*. These phytochemicals of medicinal plants have primarily reported for their medicinal value, which can be valuable folklore remedies in the treatment of cold, headache, acne, malaria and bacterial diseases.\(^{[15]}\) The plant containing phenolic compounds contributed to their antioxidative properties and thus the value of the plants are in folklore medicine. Phenols have been practicing in the preparation of some antimicrobial agents such as dettol and cresol. Both plants are widely used regularly among many tribes in Africa for the treatment of various diseases. For instance, saponins proved as hypotensive and cardiodepressant properties\(^{[16]}\), which are helpful for the management of heart failure and cardiac myopathy.\(^{[17]}\) The occurrence of saponins in various extracts leaves of *F. vasta* might play a role in the cardioprotective potential. alkaloids have the potential of anti-hyperglycaemic and anti-inflammatory activities.\(^{[17]}\)
Table 1: Phytochemical investigation of leaves, stem and fruits of Ethiopian fig plant using aqueous, methanol, hexane and ethanol solvents.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Ficus vasta Forssk.</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aqueous</td>
<td>Methanol</td>
<td>Hexane</td>
<td>Ethanol</td>
<td>Aqueous</td>
<td>Methanol</td>
<td>Hexane</td>
<td>Ethanol</td>
<td>Aqueous</td>
<td>Methanol</td>
<td>Hexane</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Protein and aminoacids</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Total phenols and Tannins</td>
<td>++</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+++ = appreciable amount (positive within 5 mins.); ++ = moderate amount (positive after 5 mins. but within 10 mins); + = trace amount (positive after 10 mins. but within 15 mins); - = completely absent.

CONCLUSION

The results of phytochemical analysis showed the leaves, fruit and stem extracts of *F. vasta* indicates their potential as a source of bioactive principles that may supply drugs for modern medicines. Further studies are therefore required to validate their antimicrobial, antihyperglycemic, anti-inflammatory and antihelminthic activities. In addition, extraction, cleansing and categorization of the active principles are required to make the plant has novel interesting studies.
ACKNOWLEDGMENT
We sincerely acknowledge to the Staff of the Biomedical Sciences, Jimma University, Jimma, Ethiopia who really gave support and cooperation in the study.

REFERENCES


