

Screening of Phyto-chemical compounds from hydro-ethanolic and ethanolic leaf and bark extracts of Terminalia arjuna and Syzygium cumini

Dr. Swaati Sharma , Priya Vishnoi

Department of Agriculture, Food and Biotechnology,
Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India.

Abstract: Medicinal plants have many phyto-chemicals or secondary metabolites which are used to treat many human diseases (oxidative stress diseases and neurodegenerative diseases). Neurodegenerative diseases include stroke, Parkinson's disease and Alzheimer's disease and oxidative stress diseases such as inflammatory bowel diseases, retinal ischemia and cardiovascular diseases. In this study, different parts of Terminalia arjuna and Syzygium cumini plants (mainly leaves and bark) are used for screening of bioactive compounds. Plant parts are extracted with ethanolic and hydro-ethanolic (50%) solvents by Soxhlet apparatus extraction method and screened for the presence of secondary metabolites namely alkaloids, flavonoids, saponins, steroids, tannins, glycosides, phenol, terpenoids and fatty acids.

In this research work, We found that selected plants are good source of various bioactive compounds. However, both leaves and bark of Terminalia arjuna contain high amount of phyto-chemicals than Syzygium cumini. From this study, We concluded that both plants are good source of secondary metabolites and can be used as medicinal source for the treatment of various diseases.

KEYWORDS: Terminalia arjuna, Syzygium cumini, secondary metabolites, phyto-chemicals, bioactive compounds.

1. INTRODUCTION:

Since ancient times, secondary metabolites have ability to prevent chronic diseases far away their role as dietary antioxidants (1). According to World Health Organisation (WHO), medicinal plants can be good source to obtain variety of drugs (2). Generally herbal drugs are less expensive, safe, and easily available and rarely have side effects. These are widely used in human therapy, veterinary, scientific research and agriculture etc. A large number of phyto-chemicals belong to various chemical classes have prohibitory effects on all types of microorganism *in vitro*. Medicinal plants contain many bioactive compounds and are concentrated in the endangered parts of the plants such as leaves, bark, flowers, fruit and skin, used for various medicinal purposes (3,4, 5). Secondary metabolites have their role in several health benefits and provide a source of medicine since historic times. Traditional plants are helpful in world's pharmaceuticals and serve as starting material in drug development. It has been reported that these natural secondary metabolites may reduce the risk of cardiovascular diseases, oxidative stress diseases and neurodegenerative diseases (1).

The plants chosen for this study are Terminalia arjuna (*T.arjuna*) and Syzygium cumini (*S. cumini*). *T.arjuna* is large sized tree to 30 m tall, belongs to *Combretaceae* family. This large an evergreen tree appeared in hot season (February to April), found in South Asian region having wide spectrum of biological activity. The bark of *T. arjuna* have been reported for anti-dysenteric, antipyretic, astringent, cardiogenic, lithotriptic, anticoagulant, hypolipidemic, antimicrobial (6) and antiuremic (7) activities. Many useful phytochemicals have been isolated from *T.arjuna* including terpenoids for cardiovascular properties, tannins and flavonoids for its antimicrobial and anticancer properties etc (8).

On the other hand, *S.cumini* is commonly known as jambolan or java plum, belongs to family *Myrtaceae*. Plants of this family are rich in volatile oils which are used in medicines (9). Fruits of this family are used as edibles and as traditional medicines in divergent ethno-botanical practices throughout the tropical and subtropical world (10). The fruits are berries (edible), 1.5 to 3.5 centimeters long, dark purple and nearby black color contain single large seed (11, 12). The fruit is sweet, mildly sour and astringent in taste and contains raffinose, glucose, fructose (13), citric acid, mallic acid (14), gallic acid, anthocyanins (15).

2. MATERIALS AND METHODS:-

2.1 COLLECTION OF PLANT MATERIAL:

The leaves and bark of *T.arjuna* and *S.cumini* were procured from the botanical garden of Jayoti Vidyapeeth Women's University.

2.2 EXTRACTION OF PLANT MATERIAL:

Fresh plant materials were washed with running tap water and dried at room temperature. After washing and drying, leaves and barks of the plants were grinded using grinder to make powder. The powder was then subjected to successive extraction with different solvents (50% hydro-ethanol and ethanol) using Soxhlet apparatus method. The hydro-ethanolic and ethanolic extracts were prepared by taking 25 grams of each powdered sample and soaked in 250 ml of 50% hydro-ethanol or ethanol solvent system. The collected extracts were stored in calcium carbonate at very low temperature and then taken up for further research work.

2.3 PHYTOCHEMICAL ANALYSIS:

Chemical tests were carried out on 50% hydro-ethanolic and ethanolic extracts of aerial parts of *T.arjuna* and *S.cumini* using standard procedures to identify the constituents (16,17,18).

Test for Glycosides: For the estimation of glycosides, extract was diluted with solvent by which extract was extracted from plant materials. 2ml of diluted plant extract was mixed with 2ml of chloroform and 2ml of sulphuric acid then shaken well. Reddish brown color indicated the presence of steroidal ring (glycone portion of glycosides).

Test for Fatty acids: 0.5gm of plant extract was mixed with 5ml of ether and then extract was evaporated on filter paper and dried the filter paper. The transparency appeared on filter paper indicated the presence of fatty acids.

Test for Flavonoids: Alcoholic solution of extract was mixed with few drops of 10% lead acetate. Yellow precipitate indicated the presence of flavonoids.

Test for Phenol: Diluted extract was mixed with few drops of ferric chloride solution. Appearance of bluish black color showed the presence of phenol.

Test for Resins: 0.5gm of plant extract was diluted with 10 ml of water and then shaken for 5 min. Turbidity formed in the solution indicated the presence of resins.

Test for Saponins: Diluted 5ml extract was stirred with 5ml of distilled water and then warmed in a test tube. Stable formation of foam indicated the presence of saponins.

Test for Steroids: Diluted 2ml of extract was stirred with 2ml of chloroform and 2ml of conc. Sulphuric acid. Red color produced in the lower chloroform layer indicated the presence of steroids.

Test for Tanins: 2ml of extract was mixed with 2ml of DW and then 2 or 3 drops of ferric chloride was mixed to the solution. Green precipitate indicated the presence of tannins.

Test for Terpenoids: To identify the presence of terpenoids, Salkowski method was used. 0.5 gm plant extract was mixed with 2ml of chloroform and 3ml of conc. Sulphuric acid to form a layer. Reddish brown color indicated the presence of terpenoids.

QUALITATIVE ESTIMATION OF PHYTOCHEMICALS:-

TABLE NO:- 1

NAME OF PHYTO-CHEMICALS	NAME OF MEDICINAL PLANTS							
	TERMINALIA ARJUNA				SYZYGIUM CUMINI			
	LEAVES AND BARK EXTRACTED WITH 50% HYDRO-ETHANOL		LEAVES AND BARK EXTRACTED WITH ETHNOL		LEAVE AND BARK EXTRACTED WITH 50% HYDRO-ETHANOL		LEAVES AND BARK EXTRACTED WITH ETHANOL	
	Leaves	Bark	Leaves	Bark	Leaves	Bark	Leaves	Bark
Saponins	++	+++	++	++	++	++	+++	++
Steroids	++	+++	++	+	+++	++	++	+
Flavonoids	+++	++	+++	++	++	+++	+++	++
Phenols	+++	++	+++	++	+++	+++	+++	++
Resins	+++	+	++	+	+++	+++	+	++
Tanins	+++	++	+++	++	++	++	++	++
Terpenoids	+++	++	+++	++	++	+	++	++
Fatty acids	+++	-	+++	-	-	-	-	-
Glycosides	+++	+++	+++	+++	+++	++	+++	++

+++ High concentration, ++ Moderate concentration, + Low concentration, - Absence.

3.RESULT AND DISCUSSION :

The qualitative estimation of phyto-chemicals of plants *T.arjuna* and *S. cumini* exhibited the presence of various bioactive components such as glycosides, saponins, flavonoids, phenol, resins, tannins, terpenoids, fatty acids. From this study, I found that *T.arjuna* contains high amount of phyto-chemicals than *S.cumini*. Different plant parts with different solvents were screened to check the presence of various secondary metabolites. Results have been shown in table no.1.

Leaves of *T.arjuna* extracted with 50% hydro-ethanol contain high (+++) amount of flavonoids, phenols, resins, tannins, terpenoids, fatty acids and glycosides and moderate (++) amount of saponins and steroids, whereas, the bark extracted with the same solvent contains high (+++) amount of saponins, steroids and glycosides, moderate (++) amount of flavonoids, tannins, terpenoids and phenols, low (+) amount of resins and fatty acids are absent(-).

In case of leaves extracted with ethanol contain high (+++) flavonoids, phenol, tannins, terpenoids, fatty acids and glycosides, moderate (++) saponins, steroids and resins, whereas, the bark extracted with the same solvent contains high (+++) glycosides, moderate (++) saponins flavonoids, phenols, tannins and terpenoids, low (+) steroids and resins, fatty acids are absent(-). The bark of *T.arjuna* functions as a diuretic in cirrhosis of liver and gives relief in symptomatic hypertension (19). Its leaves have been reported to have analgesic and anti-inflammatory properties (20). *T.arjuna* is also known as cardiotonic since historic times (21). The bark of *T.arjuna* and juice of it is used as antacid by the people live in malkangiri district of orissa

(22). Powder of bark is boiled with water and inhaled to cure headache and kill bacteria in teeth (23). Bark ash is used to treat snakebite and scorpion sting (21). The bark of *T.arjuna* is also used to treat fever and high blood pressure in south surguja district of Madhya Pradesh (24). It has been proven that *T.arjuna* and its species have potential to treat HIV (25).

On the other hand, leaves of *S.cumini* extracted with 50% hydro-ethanol contain high (+++) steroids, phenols, resins and glycosides, moderate (++) flavonoids, tannins, terpenoids and saponins, fatty acids are absent(-), whereas, bark extracted with the same solvent contains high (+++) flavonoids, phenols and resins, moderate (++) saponins, steroids, tannins and glycosides, low (+) terpenoids and fatty acids are absent (-).

However, In case of leaves extracted with ethanol contain high (+++) saponins, flavonoids, phenols and glycosides, moderate (++) steroids, tannins and terpenoids, low (+) resins and fatty acids are absent (-), whereas the bark extracted with the same solvent contains moderate (++) saponins, flavonoids, phenols, resins, tannins, terpenoids and glycosides, low (+) steroids and fatty acids are absent (-). The fruits of *S.cumini* having medicinal properties, used to treat cough, diabetes, inflammation, dysentery and ringworm (10). The fruit (mainly seeds) of *S.cumini* is also claimed to contain an alkaloid, jambosine, and a glycoside, jambolin or antimellin, which stop the diastatic conversion of starch into sugar (26). They are also rich in flavonoids (a well known antioxidant), which accounts for the scavenging of free radicals (27, 28). Jambolan also have phenolics with antioxidant activity (29) and is rich in protein and calcium. Java plums are also rich in mineral salts, sugar, vitamin C, PP which boosts the beneficial effects of vitamin anthocyanins and flavonoids (30). Different parts of the jambolan have been reported for its antioxidant, anti-inflammatory, neuropsychopharmacological, anti-microbial, anti-bacterial, anti-HIV, antileishmanial and antifungal, nitric oxide scavenging, free radical scavenging, anti-diarrheal, antifertility, anorexigenic, gastroprotective and anti-ulcerogenic and radioprotective activities (31).

Phyto-chemicals in our diet promote the health (1). Phyto-nutrients are related with the prevention of recognized chronic diseases including cardio vascular diseases (CVDs), cancer, osteoporosis, diabetes and vision diseases that are mainly terrible in Western countries. These diseases are mostly attributed to a high-sugar and high-fat diet, smoking, lack of exercise and other unhealthy lifestyle (32). Interaction of carcinogens or oxidants with DNA causes many types of cancers. The powerful antioxidant ability of phyto-nutrients can reduce this damage (33). Different parts of plant are also used to treat various diseases such as diabetes, blisters in mouth, cancer, colic, diarrhea, digestive complaints, dysentery, piles, pimples and stomachache (34).

Phyto-chemicals, found in bark and leaves of *T.arjuna* and *S.cumini*, play important roles to treat various diseases. Tannins have the ability to treat sore throat, wound healing, anti-diarrhoea and antihaemorrhagic agent (35, 36). Tannins have also been studied for the antimicrobial degradation of dietary proteins or semen (37). Flavonoids can inhibit the initiation, promotion and progression of tumors. Phenols and flavanoids, both are strong water soluble antioxidants (38). These phenolic compounds also have some biological properties such as anti-apoptosis, anti-septics, anti-carcinogen, anti-ageing, anti-inflammation, anti-atherosclerosis, improvement of endothelial function and cardiovascular protection (36). These phenolic compounds also have ability to inhibit angiogenesis and cell proliferation activities (39). Terpenoids have carboxylic acid group which is responsible for the activity of organic extracts (40,16). Glycosides are present in all the hydro-ethanolic and ethanolic extracts of *T.arjuna* and *S.cumini* which lower the blood pressure (41). Saponins, found in hydro-ethanolic and ethanolic extracts of both the plants are known to possess reduction in cholesterol level and can manage cardiovascular diseases in humans (37). Saponins also have been used as emulsifying agents and having anti-fungal (42), anti-inflammatory activity (43), coagulating and precipitating property in red blood cells. Steroids also have been found in these medicinal plants, previously reported for its antibacterial activity (44) and their association with such compounds as sex hormones (45). Fatty acids, found in hydro-ethanolic and ethanolic extracts of *T.arjuna* leaves are previously reported for their anti-fungal and anti-bacterial activities (46). Resins are also present in these traditional medicinal plants, used for the treatment of arthritis, wound healing and have anti-inflammatory, anti-microbial, anti-tumor, anti-hyperlipidemia properties (47).

4. CONCLUSION:

From this study, it can be concluded that these species may be used for drug development in pharmaceutical companies. Different parts (leaves and bark) of both plants are good source of phyto-chemicals which have anti-fungal and anti-microbial properties.

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