WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 15, 1058-1065. Research Article ISSN 2277– 7105

INVESTIGATION OF PHYTOCHEMICALS FROM DIFFERENT PARTS OF *Aegles marmelos* L.

Rajeswari G.* and Nagarajan. N.

Department of Ancient science, Faculty of Science Tamil University, Thanjavur.

Article Received on
02 Oct. 2017,

Revised on 23 Oct. 2017,
Accepted on 14 Nov. 2017

DOI: 10.20959/wjpr201715-10161

***Corresponding Author**
Rajeswari G.
Department of Ancient science, Faculty of Science Tamil University, Thanjavur.

ABSTRACT

In the present investigation suggested that the phytochemicals (qualitative and quantitative) analysis was carried out from leaves, bark, and fruits of *Aegle marmelos*. It was alkaloids, flavonoids, phenols, reducing sugars, saponins, steroids, tannins, terpenoids, glycosides and anthiroquinone were qualitatively analyzed and alkaloids, flavonoids, phenols, reducing sugars, saponins, terpenoids, steroids, and glycosides were quantitatively excellent of phytochemicals estimated. Among the three parts, fruit was excellent results of plant in both qualitative and quantitative phytochemicals when compared to leaf and bark samples.

KEYWORDS: Phytochemicals, *Aegles marmelos*, Fruit, Leaf, Stem.

INTRODUCTION

Plant derived compounds got an increasing interest throughout the world as they possess potent, less or no toxic pharmacological compound, economic viable, safer and more dependable process. (Prashant *et al*., 2008). About 70 – 95% of the world population is relying on traditional medicines or traditional therapies where the whole or parts of plants is used as medicine. (Robinson, 2011). Drug resistances in microorganism have become an unsolvable problem and treating an infectious disease with the existing drugs is becoming less used. This situation, truly made researchers with discover drug from various sources, one such source is plant based drugs. *Aegle marmelos* belonging to the family *Rutaceae* is one such plants where all the parts of the plant are known to possess various pharmacologically active compounds. The global demands for bi-products of traditional medicinal plants have been increasing nowadays. The procuring of medicinal plants is mellow, cost effective, enormous pharmacological active principles, and it is in practices as a home remedy for many

human diseases when compared to the modern medicine in used, which are adulterated and have subjected to many side effects. (Saet *et al*., 2007). To assess the multi-infection targeted by the antibiotic-resistant microorganisms nowadays have urged scientists in search of new drugs with less side effects. (Kapoor *et al*., 2015) The plants have the ability to synthesize invaluable source of bioactive compounds which possessing antimicrobial potential and therapeutic effects. (Mini, *et al*., 2015). The world wide researchers are still having the thirst in search of new antimicrobial compounds from plants (El-Seedi, 2002; Dandjesso *et al*., 2012). Since *Cassia alata*, a traditional medicinal plant has been very effective for many human ailments and to treat many types of skin infections (Igoli *et al*., 2005). The leaf extracts of credential for the medicinal effects on intestinal parasitoids, syphilis, hemorrhoids, linguinal hernia, constipation and diabetes (Jadhav *et al*., 2015). Many researchers have reported that the compounds are isolated from *Aegles marmelos* previously such as hydroxyl anthraquinones, glycosides, chrysophanic acid and kampferin (Mirzaei *et al*., 2015) possessing many biological functions.

MATERIALS AND METHODS

Identification and collection of plant

The *Aegles marmelos* plant parts were collected from the local area of Thanjavur, Tamilnadu, India. The taxonomy of the plant was authenticated with St. Joseph;s College, Trichy.

Extract preparation

The leaf, bark, and fruits of the selected plants for the study were taken (200gm) and blended with mixer grinder, extracted with 100% methanol and filtered after 2 hours. The plant residue was again extracted with the addition of 80% methanol and left standby for 24 hours. It was filtered and the procedure was repeated every 24 hours, until the sample become pale. All the filtrates were combined and concentrated in a rotary evaporator at 45˚C. The extracts were refrigerated in a sterile bottle (Betoni, 2006).

Phytochemical analysis

The qualitative phytochemical analysis of the extracts was performed by following the protocol (Trease *et al*., 1989).

Tannins: To 200mg of plant extract is added with 10ml of distilled water, the mixture is boiled and filtered after cooling. Few drops of $FeCl_3$ are added to the filtrate Blue-black precipitate was observed in the presence of Tannins.

Alkaloids: 10ml of methanol added with 200mg of the plant extract, it was boiled and filtered after cooling. To that filtrate added 1%HCl followed by a few drops of Dragendorff reagent, brownish red precipitate was observed.

Saponins (Frothing test): 5 ml distilled water and 0.5 mg of the plant extract were shaken vigorously for 2 minutes and the durable foam indicates the presence of saponins.

Cardiac Glycosides: 1 ml of glacial acetic acid containing a few drops of $FeCl_3$was added to 2mg of the plant extracts. The above mixture is treated with concentrated sulphuric acid (H_2SO_4), greenish blue colour depicting positively.

Steroids (Liebermann-Burchard reaction): 10 ml of chloroform and acetic anhydride was added in the ratio 1:1 to 200 mg of the plant extract results in the development of blue-green ring.

Terpenoids (Salkowski test): 2 ml of $CHCl_3$ and 3 ml of concentrated sulphuric acid (H_2SO_4) were added to 200 mg of the plant extract, reddish brown development signified as terpenoids.

Flavonoids: 5 ml of dilute ammonia, followed by concentrated H_2SO_4 was added to the plant extract deep yellow coloration indicates flavonoids.

Reducing Sugars: Few drops of Fehling's solution A and B were added to the plant extract; an orange red precipitate was observed which suggests the presence of reducing sugars.

Quantitative estimation of phytochemicals

Estimation of Flavonoids

The flavonoid content was determined by the standard method. 1 mg of the plant extract was dissolved in1ml of 80% ethanol. To 0.5ml of the extract, added 80% of 4.3ml ethanol, 10% of 0.1ml aluminium nitrate and 1M of 0.1ml potassium acetate. The test tubes are standing by for 40 minutes at room temperature. The absorbance was read at 415 nm. The total flavonoid content in the extract was determined as µg standard quecertin equivalent.

Estimation of Tannins

The estimation of tannins with minor modifications was performed by the method, (Swain *et al*., 1979). 1 ml of the sample extract was treated with 2.5 ml of Folin-Denis reagent, 10ml of

17 % Na_2CO_3 and 20 ml of distilled water were kept in room temperature for 20 minutes, the colour development of bluish green was observed and the absorbance was measured at 760 nm and calculated the amount tannin by comparing it with standard curve.

Estimation of Total Phenols

Folins-Ciocalteau method was followed to determine the total phenolic content in the polyphenolic extract. Mix 2.5ml of freshly prepared Folin-Ciocalteau reagent with 3ml of 2% sodium carbonate and 0.5ml of the extract left for 2hours at room temperature. It was read at 760 nm and the results were expressed in gallic acid equivalents (mg/g; mg gallic acid/g dried extract) (Obodoni *et al*., 2001).

RESULT AND DISCUSSION

The extraction of phytochemicals with methanolic solvent was alkaloids, flavonoids, phenols, saponins, steroids and anthoquinone were present in the partially purified extract of *Aegles marmelos* leaves were analyzed whereas bark was alkaloids, steroids, phenols, and glycosides recorded respectively. In the part of fruits was flavonoids, phenols, saponins, tannins, terpenoids, glycosides, and anthroquinone, phytochemicals represented by *Aegles marmelos* Plant (Table-1).

The bearing of various phytoconstituents in the extract like flavonoids, tannins, alkaloids, saponins, terpenoids, cardiac glycosides, and reducing sugars were present in the partially purified extract of *Cassia alata* (Idu, *et al*., 2007) reported that the secondary bioactive compounds such as phenol, tannins, and flavonoids were promising source of *Cassia alata*.

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloid, flavonoid, tannins, phenolic compounds, saponins and phytosterols (Britto and Sebastian, 2012). The presence of alkaloisds, saponin, flavonoids, phenolic compounds, tannins, phytosterol and terpenoids are used in analgesic, and antiplasmodic and bacteriocidal activities (Stary, 1998). Thus the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development (Nithyadevi *et al*., 2015).

The estimation of phytochemicals in the flower extracts of C*assia alata* contain a remarkable amount of flavonoids, phenolics and tannin contents recorded. The partially purified extract of *Cassia alata* contained the highest quantity of flavonoids (72.9±0.061), phenols

(49.4±0.04), and tannins (18.12±0.30). The biological action of the plant extract cannot be ascertained only by the result of phytochemical studies.

The results revealed the presence of pharmacologically active compound such as alkaloids, flavonoids, terpenoids, saponins and phlobatannins in leaves, fruit pulp and rind. (Samrot *et al*., 2010; Abirami *et al*., 2013; Joshi *et al*., 2009). Presence of alkaloids, Flavonoids, Phenolic Compounds, Steroids, Saponins and Xanthoproteins in invitro generated *Aegle marmelos* was detected (Kumari *et al*., 2011). Quantitative phytochemicals of leaf was 2.01, 1.30, 1.14, 1.60, 0.85, 0.65, 1.32, 1.0.2, and 1.62 mg/g with alkaloids, flavonoids, phenols, reducing sugars, streriods, tannins, terpenoids, terpenoids, glycosides, anthroquione recorded respectively whereas in the bark samples of 4.15, 1.25, 1.50, 1.15, 2.65, 1.32, 1.42, 1.33, and 1.00 mg/g with respective phytochemicals represented from the plant. The *Aegle marmelos* fruit with methanolic extract was 5.70, 2.16, 2.05, 1.85, 1.30, 1.10, 2.30, 3.10 and 4.30 mg/g with alkaloids, flavonoids, phenols, reducing sugars, streriods, tannins, terpenoids, and glycosides estimated. Among the three parts, the fruit of *Aegle marmelos* was found to be maximum phytochemicals when compared to other part of the leaf and bark of the plant. (Table-2).

Table 1: Qualitative phytochemical analysis of *Aegle marmelos.*

S. No	Name of the Phytochemicals	Leaves	Bark	Fruit
1	Alkaloids	+	+	+
2	Flavonoids	+	+	+
3	Phenols	+	+	+
4	Reducing sugar	+	-	+
5	Saponin	+	+	+
6	Steroids	+	+	+
7	Tannin	+	+	+
8	Terpenoids	+	+	+
9	Glycosides	+	+	+
10	Anthroquinone	+	+	+

(++) present (--) absent

Table 2: Studies on the quantitative analysis of *Aegle marmelos.*

S.No	Name of the phytochemicals	Quantity of phytochemicals (mg/g)		
		Leaves	Bark	Fruit
1	Alkaloids	2.01	4.15	5.70
2	Flavonoids	1.30	1.25	2.16
3	Phenols	1.14	1.50	2.05
4	Reducing sugars	1.60	1.15	1.85
5	Saponins	0.85	2.65	1.30
6	Tannins	0.67	1.32	1.10
7	Terpenoids	1.32	1.42	2.30
8	Steroids	1.02	1.33	3.20
9	Glycosides	1.62	1.00	4.30
10	Anthoquinone	1.01	0.85	1.45

CONCLUSION

The results obtained in the present investigation are encouraging and can be used as reference data for the standardization of leaves of *Aegle marmelos* (L.) and the formulations containing these plant leaves, bark and fruit as a main ingredient. Though this plant is very common plants having less possibility of adulteration, but to get highest efficacy of an herbal drug or its finished product, cent percent genuine plant material should be the source. All these above said characters reflect the diagnostic features of the leaves of *Aegle marmelos*. The preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Such screening experiments form a primary platform for further phytochemicals and pharmacological studies that may open the possibility of finding new clinically effective compounds. Thus, the present study has authenticated the usefulness of the *Aegle marmelos* plant for medicinal purposes.

REFERENCE

1. Abirami, H. and Kumar, PS., (2013). *Invitro* regeneration and extraction of secondary metabolites in *Aegle marmelos* (L.), *Asian Journal of Plant Science and Research*, 3(2): 99-106.
2. Betoni, JE., Mantovani, RP., Barbosa, LN., De Stasi, LC. and Junior, FA., (2006). Synergism between plant extract and antimicrobial drugs used on *Staphylococcus* diseases. *Mem Instruction Oswaldo Cruz*, 101(4): 387–390.
3. Britto, J.D. and Sebastian, S.R., (2012). Biosynthesis Silver Nanoparticles and its Antibacterial Activity against Human Pathogens. *International. Journal. Pharmacology Science*, 5: 257-259.

4. Dandjesso, C., Klotoa, JR., Dougnon, TV., Segbo, J., Ategbo, JM., Gbaguidi, F., Fah, L., Fanou, B., Loko, F. and Dramane, K., (2012). Phytochemistry and hemostatic properties of some medicinal plants sold as anti-hemorrhagic in Cotonou Markets (Benin). *Indian Journal of Science and Technology*, 5(7): 3105–3109.
5. El-Seedi, HR., Ohara, T., Sata, N., Nishiyama, S., (2002). Antimicrobial diterpenoids from Eupatorium glutinosum (Asteraceae). Journal of Ethnopharmacology, 81(2): 293–296.
6. Idu, M., Omonigho, SE. and Igeleke, CL., (2007). Preliminary investigation on the Phytochemistry and antimicrobial activity of Senna alata L. Flower. *Paki Journal Biological Science*, 10(5): 806–809.
7. Igoli, JO., Ogaji, OG., Igoli, NP. and Tor-Anyiin, TA., (2005). Traditional medicinal practices among the Igede people of Nigeria (part II). *African Journal Traditional Compliment Altern Med*, 2(2): 134–152.
8. Jadhav, BA. and Joshi, AA., (2015). Extraction and quantitative estimation of bio active component (yellow and red carthamin) from dried safflower petals. *Indian Journal of Science and Technology*, 8(16): 1–5.
9. Joshi, B., Lekhak, S. and Sharma, A., (2009). Antibacterial Property of Different Medicinal Plants: *Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum and Origanum majorana* Kathmandu University. *Journal of Science, Engineering And Technology*, 5: 143-150.
10. Kapoor, S., Ranote, PS. and Sharma, S., (2015). Bioactive Components and Quality Assessment of Jamun (*Syzygium cumini* L.) powder supplemented Chapatti. *Indian Journal of Science and Technology*, 8(3): 1–9.
11. Kumari, S.T.K., Lincy, M.P., Muthukumarasamy, S. and Mohan, V.R., (2011). Antiinflammatory activity of *Sarcostemma secamone* (l) bennet whole plant against carrageenan induced paw edema. *Bioscience Discovery*, 3(3): 288-291.
12. Mini, JJ., Christhudas, IVSN. and Gajendran, N., (2015). In-vitro anti-inflammatory activity of the fractions isolated from *Walsura trifoliata* (A. Juss.) Harms Root. *Indian Journal of Medicine and Healthcare*, 4(3): 1–5.
13. Mirzaei, A. and Rezanejad, MT., (2015). Hepatoprotective effect and antioxidant activity of aqueous cherry extract on rats. *Indian Journal of Science and Technology*, 8(22): 1–5.
14. Nithyadevi, J. and Sivakumar, R., (2015). Phytochemical Screening and GC-MS, FT-IR Analysis of Methanolic Extract Leaves of *Solanum torvum* Sw. *International Journal of Research Studies in Biosciences*, 61-66.

15. Obodoni, A. and Ochuko, P.O., (2001). Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure Applied Science*, 8(3): 203–208.
16. Prashant, K.R., Dolly, J., Singh, K.R., Gupta, K.R. and Watal, G., (2008). Glycemic properties of *Trichosanthes dioica* leaves. *Pharmarmacology. Biological*, 46(12): 894-899.
17. Robinson, M.M., (2011). Classifications, Terminology and Standards, WHO, Geneva: Xiaorui Zhang Traditional Medicines, WHO Traditional medicines: global situation, issues and challenges. 3rd Edition.
18. Saet, BL., Kwang, HC. and Su, NK., (2007). The antimicrobial activity of essential oil from *Dracocephalum foetidum* against pathogenic microorganisms. *Journal of Microbiology*, 45(1): 53–57.
19. Samrot, A.V., Mathew, A.L., Shylee Hemalatha, N. and Karunya, A., (2010). Evaluation of Bioactivity of Various Indian Medicinal Plants –An *In-Vitro* Study. *The Internet Journal of Internal Medicine*, 8(2).
20. Stary, F., (1998). The Natural Guide to Medicinal Herb and Plants. Tiger Books International, London, 12-16.
21. Trease, GE. and Evans, WC., (1989). Pharmacognosy. Brailliar Tiridal Can Macmillian Publishers, 11th edition.