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IDENTIFICATION OF PHYTO-CHEMICALS OF *SYZYGIUM CUMINI* FRUIT

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ABSTRACT

This study was carried out to identify phyto-chemicals of *Syzygium cumini* (L.) Skeels. For this, dry and powdered fruit of the tree was extracted in methanol and partitioned with *n*-hexane and chloroform. Using solvent system of chloroform: *n*-hexane (30:70), four compounds were identified on a TLC plate which were separated on a preparative TLC and further purified on HPLC followed by identification through GC-MS analysis. These compounds included ethyl iso-allocholate **(1)**, oxime-, methoxy-phenyl **(2)**, heptacosane **(3)** and octadecane **(4)**. A thorough literature survey was conducted to search for various bioactivities of the identified compounds. These compounds were found to possess antioxidant, anti-inflammatory, antimicrobial, anticancer, antifungal, antibacterial and/or antiasthma properties.

Key-words: Bioactive constituents, HPLC, Fruit, Phyto-constituents, *Syzygium cumini*.

INTRODUCTION

Syzygium cumini (L.) Skeels, family Myrtaceae, is an evergreen plant native to South-east Asia, Indian Sub-continent, Australia and tropical America (Sherif, 2019). It is highly unevenly worldwide distributed plant and is very often cultivated to subtropical and tropical regions (Plathia *et al.*, 2018). The plant produces plums that are astringent, mildly sour or sweet in flavor, commonly known as black plum, Indian blackberry, java plum, jamun, purple plum, damson plum and Jamaica (Ali *et al.*, 2016). The fruit is 1.8 to 3.2 cm long, oval shaped with a deep purple color having a hard seed inside (Chaudhary and Mukhopadhyay, 2012). The unripen fruit juice is being used in jelly, squash, vinegar and preservatives preparation (Agarwala *et al.*, 2019). It is rich in fructose, mallic acid, anthocyanins, tannins, gallic acid, glucose, citric acid, raffinose, petunidin, malvidin cyanidin and diglycosides of commercial importance (Singh *et al.*, 2018).

Traditionally, the fruit possesses effective antibacterial, antioxidant and anti-inflammatory properties (Singh *et al.*, 2016). It produces saccharides, polyphenols and proteins which have antidiabetic effects and have been in practice against diabetes for more than 100 years (Amudha *et al.*, 2018). Moreover, antimellin, glycoside, jambosine and alkaloids are also present in the fruit that convert starch into sugar with effective decrease in blood pressure and ellagic acid contents (Suryajayanti *et al.*, 2017). It is also effectively used for the cure of inflammations, ringworm, dysentery, ailments, cough, pimples, piles, stomachache, diarrhea, cancer, colic and blisters (Banerjee and Rana, 2020). The aim of this study was to identify various phyto-components in chloroform fraction of methanolic fruit extract of *S. cumini* and to collect information regarding various bioactivities of the identified compounds from literature.

MATERIALS AND METHODS

Fresh fruits of *S. cumini* were collected from Lahore, Pakistan, dried and ground to a course powder. Material was extracted in methanol for two weeks and after filtration, the solvent was evaporated on a rotary evaporator. The obtained methanolic extract was mixed in water and partitioned successively using *n*-hexane and chloroform. A number of solvent systems were tried and finally chloroform: *n*-hexane (30:70) was used on TLC plate. To isolate these compounds, silica gel plates (20 × 20 cm^2) were used in preparative thin layer chromatography (PTLC). With the help of a fine needle, the four compounds were scratched from surface of the plate. To detach the compounds from silica gel, they were dissolved in chloroform: methanol (50:50). After settle down of the silica gel, the solvent mixture with dissolved compounds was separated and evaporated at 30 °C to get the compounds. For further purification of the isolated compounds, HPLC was used. HiQ Sil C18, 4.6 × 250 mm, 5 micron column was used in HPLC. A volume 20 µL of each sample was used with 20 min run time and 0.7 mL min^{-1} flow rate. HPLC chromatograms of the four sub-fractions of chloroform fraction are shown in Fig. 1. Detection was made at wavelength 270 nm. Four major compounds were collected and identified through GC-MS analysis at 70 ev and with Rtx-5 MS column (60 m × 250 µm i.d. × 0.25 µm film thickness) using helium as a carrier gas. A thorough

literature survey was carried out to search for various bioactivities of the identified compounds (Iftikhar *et al.*, 2019).

RESULTS AND DISCUSSION

S. cumini fruit confer vital compounds that have been used traditionally to cure various diseases (Jagetia and Baliga, 2002). Previously, many pharmacological studies were carried out to explore their therapeutic potential against the pathogenic microbes (Migliato, 2005; Braga *et al.*, 2007). Entire plant parts warrant the most significant phytoconstituents like oxalic acid, geranyl acetone, malieic acid, tannins, ellagic acid, vernolic acid, stearic acid, guaicol, gallic acid, cineole, oleanolic acid, pinocarvone, isoquercetin, crategolic acid, cynidin glycoside, mycaminose, isorhamnetin 3-O-rutinoside, essential oils, myricetin, flavonoids, quercetin, gallotannin, betulinic acid, ellagic acid, ellagitannin, kaempferol, syzygiresinol, ß-sitosterol, ß-sitosterol-D-glucoside, friedelan3-a-ol and friedelin with antibacterial, antimicrobial, antifungal, antineoplastic, antipyretic, antidiarrheal, hypoglycemic, hypolipidemic, antioxidant, anti-inflammatory, anti-aging, chemo preventive, antitumor, antidepressant and gastroprotective properties (Ahn *et al.*, 2005; Meragelman *et al.*, 2005; Lima *et al.*, 2007). Recent studies have shown that fruit pulp is enriched with nutritious contents and minerals like calcium, potassium, phosphorous, sodium, zinc, iron, thiamine, ascorbic acid, niacin, mannose, fructose, maltose, galactose, alanine, glutamine, cysteine, tyrosine, asparagine, cinnamyl acetate, cinnamaldehyde, chrysanthemin, nerol, linalool oxide, phenylethanol and phenylpropanal (Benherlal and Arumughan, 2007).

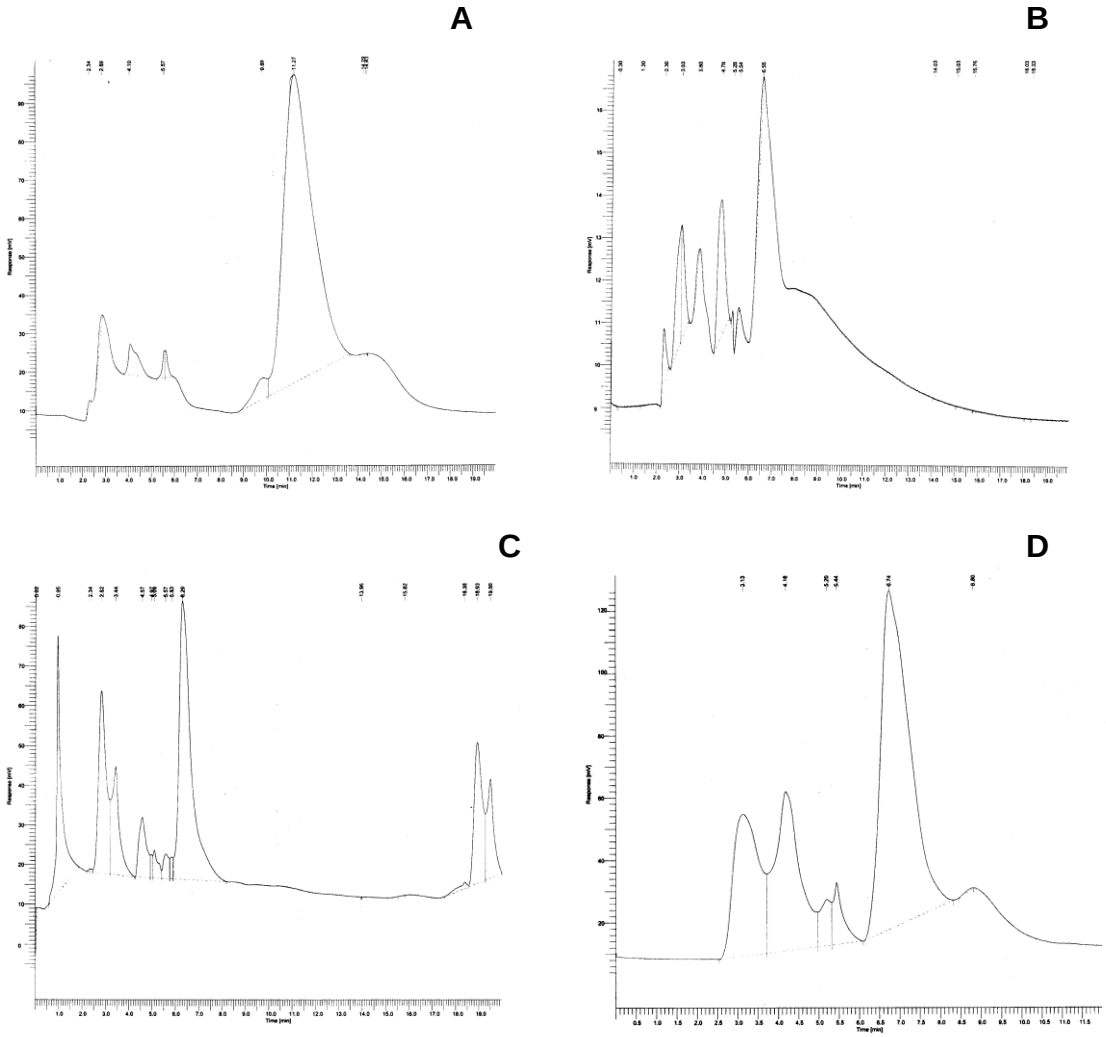

Fig. 1. HPLC chromatograms of sub-fractions of chloroform fraction of methanolic fruit extract of *Syzygium cumini* isolated by using PTLC.

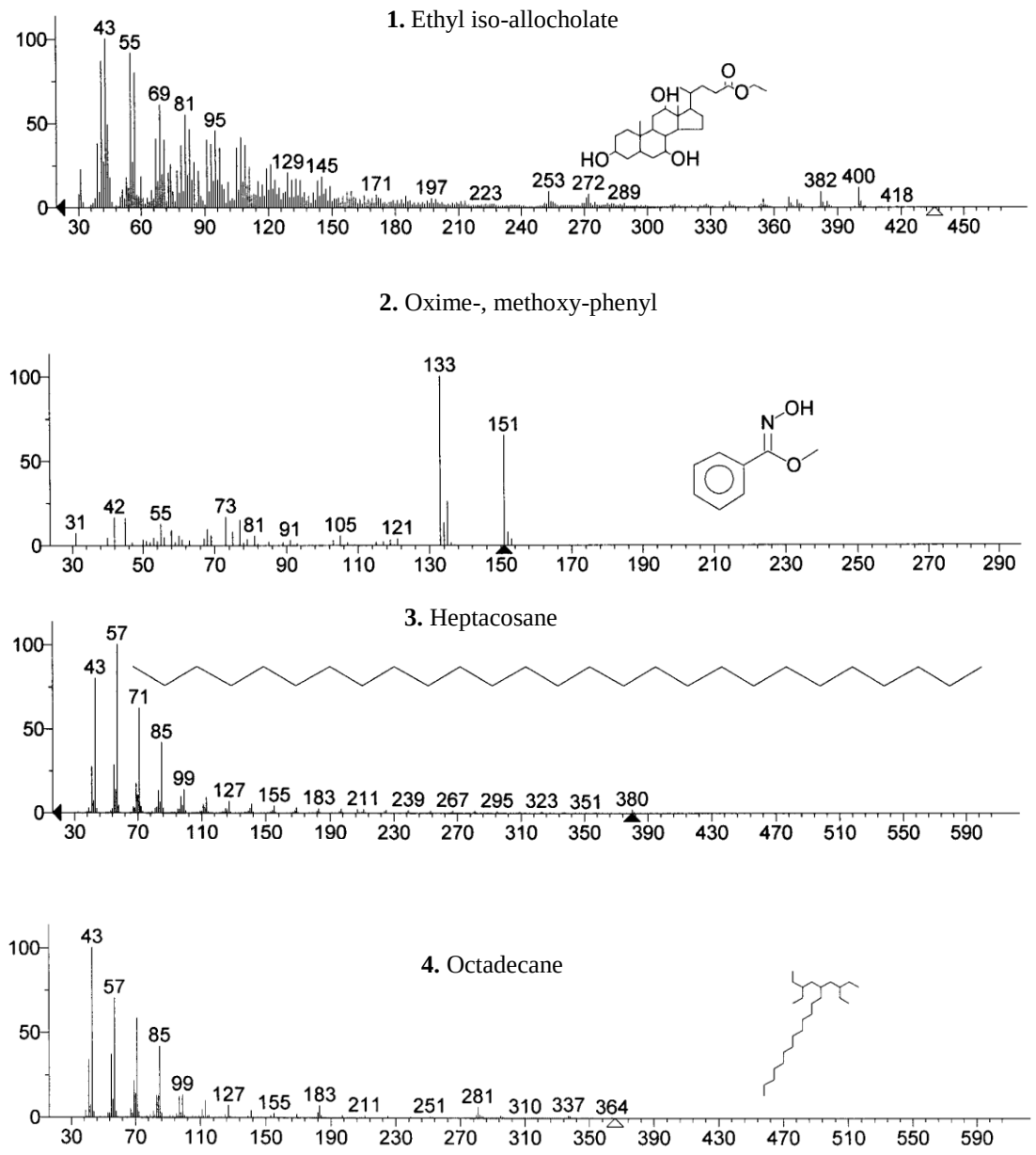

Fig. 2. Structures of compounds **1–4** isolated from chloroform fraction of methanolic fruit extract of *S. cumini.*

Table 1. Bioactivity of components of chloroform fraction of methanolic fruit extract of *S. cumini*.

Comp. No.	Names of compounds	Bioactivity	Reference
1	Ethyl iso-allocholate	Anti-inflammatory, antimicrobial, antiasthma, anti-arthritic, anticancer and diuretic	Hugar and Londonkar (2017); Sosa *et al*. (2016); Muthulakshmi *et al*. (2012); Daffodil *et al*. (2012)
2	Oxime-, methoxy-phenyl	Antioxidant, antifungal, antibacterial and antimicrobial	Altaee *et al.* (2017); Al-Jassaci *et al.* (2016); Altameme *et al*. (2015)
3	Heptacosane	Antioxidant and antifungal	Shobier *et al.* (2016); Vaithiyanathan and Mirunalini (2015)
4	Octadecane	Antimicrobial	Arora *et al.* (2017); Ozdemir *et al*. (2004)

Four compounds were identified in the present study. The first compound was ethyl iso-allocholate (**1**) with formula $C_{26}H_{44}O_5$ and molecular weight 436 (Fig. 2A). It was previously isolated from *Euphorbia lathyrism*, a Chinese medicinal plant used traditionally as a substitute to synthetic chemical constituents. In nature, it is a steroid and possesses strong anti-inflammatory, anti-arthritic, antiasthma, antimicrobial, anticancer and diuretic activities (Sosa *et al.,* 2016). The second isolated compound was oxime-, methoxy-phenyl (**2**) with molecular weight 151 and molecular formula $C_8H_9NO_2$ (Fig. 2B). It was previously identified through GC-MS analysis of methanolic leaf extract of *Urtica dioica*. This compound exhibited antimicrobial and antioxidant properties (Altameme *et al*., 2015). Moreover, it was tested against the fungal pathogenic strains namely *Fusarium* sp., *Mucor* sp., *Candida albicans*, *Penicillium expansum*, *Aspergillus flavus*, *A. fumigatus*, *A. niger* and *A. terreus*. It was found to be highly effective in reducing the growth of *A. fumigatus*, *A. terreus*, *A. flavus* and *Fusarium* sp. (Altaee *et al.*, 2017). Al-Jassaci *et al.* (2016) also worked on compound **2** and proved that it is an antibacterial against several clinical pathogenic bacterial strains *viz. Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas eurogenosa*, *Staphylococcus epidermidis*, *Streptococcus pneumonia*, *Klebsiella pneumonia* and *Proteus mirabilis* (Table 1).

The third identified compound was heptacosane (**3**) with formula $C_{27}H_{56}$ and molecular weight 380 (Fig. 2C). It was also isolated by Vaithiyanathan and Mirunalini, (2015) from the methanolic extracts of *Pergularia daemia* with antioxidant properties. Moreover, it was tested against *A. flavipes*, *F. oxysporum*, *F. solani* and *C. albicans* with promising antifungal activity (Shobier *et al*., 2016). The forth isolated compound was octadecane (**4**) with formula $C_{26}H_{54}$ and molecular weight 366 (Fig. 2D). It is a known hydrocarbon and was previously isolated from a nutritious grass *Cenchrus setigerus* hexane extract. It was reported very effective in inhibiting the growth of some plant pathogens (Ozdemir *et al.*, 2004; Arora *et al*., 2017). The present study enlightens that the four compounds isolated from chloroform fruit extract of *S. cumini* through TLC followed by HPLC possess antioxidant, anti-inflammatory, antimicrobial, anticancer, antifungal, antibacterial and antiasthma properties.

REFERENCES

Agarwala, P., P.K. Gaurb, N. Tyagia, D. Purib, N. Kumarc and S.S. Kumard (2019). An overview of phytochemical, therapeutic, pharmacological and traditional importance of *Syzygium cumini*. *Asian Journal of Pharmacognosy*, 3: 5-17.

Ahn, Y.J., H.S. Lee, H.S. Oh , H.T. Kim and Y.H. Lee (2005). Antifungal activity and mode of action of galla rhois-derived phenolics against phytopathogenic fungi. *Pesticide Biochemistry and Physiology*, 81: 105-112.

Ali, G., V. Singh, A.K. Singh, S.S. Gautam and R.K. Prasad (2016). Evaluation of antihyperglycemic activity and phytochemical screening using bark of *Syzygium cumini*. *Asian Journal of Plant Science and Research*, 6: 80-86.

Al-Jassaci, M.J., G.J. Mohammed and I.H. Hameed (2016). Secondary metabolites analysis of *Saccharomyces cerievisiae* and evaluation of antibacterial activity. *International Journal of Pharmaceutical and Clinical Research*, 8: 304-315.

Altaee, N., M.J. Kadhim and I.H. Hameed (2017). Detection of volatile compounds produced by *Pseudomonas aeruginosa* isolated from UTI patients by gas chromatography-mass spectrometry. *International Journal of Current Pharmaceutical Review and Research*, 7: 8-24.

Altameme, H.J., M.Y. Hadi and I.H. Hameed (2015). Phytochemical analysis of *Urtica dioica* leaves by fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*, 7: 238-252.

Amudha, S., P.K. Manna and N.S. Jeganathan (2018). Evaluation of anti-diabetic activity of *Syzygium cumini* extract and its phytosome formulation against streptozotocin-induced diabetic rats. *The Pharma Innovation Journal*, 7: 603-608.

Arora, S., G. Kumar and S. Meena (2017). GC-MS analysis of bioactive compounds from the whole plant hexane extract of *Cenchrus setigerus* Vahl. *Pharma Science Monitor*, 8: 137-146.

Banerjee, A. and D. Rana (2020). Leaf spot disease of *Syzygium cumini* caused by *Neopestalotiopsis clavispora* newly reported in India. *Plant Disease*, 104: 984.

Benherlal, P.S. and C. Arumughan (2007). Chemical composition and *in vitro* antioxidant studies on *Syzygium cumini* fruit. *Journal of the Science of Food and Agriculture*, 87: 2560-2569.

Braga, F.G., M.L.M. Bouzada, R.L. Fabri, M.O. Matos, F.O. Moreira, E. Scio and E.S. Coimbra (2007). Antileishmanial and antifungal activity of plants used in traditional medicine in Brazil. *Journal of Ethopharmacology*, 111: 396-402.

Chaudhary, B. and K. Mukhopadhyay (2012). *Syzygium cumini* (L.) Skeels: A potential source of nutraceuticals. *International Journal of Pharmacy and Biological Sciences*, 2: 46-53.

Daffodil, E.D., F.K. Uthayakumari and V.R. Mohan (2012). GC-MS determination of bioactive compounds of *Curculigo orchioides* gaertn. *Science Research Reporter*, 2: 198-201.

Hugar, A.L. and R.L. Londonkar (2017). GC-MS profiling of bioactive components from aqueous extract of *Pterocarpus marsupium*. *International Journal of ChemTech Research*, 10: 557-564.

Jagetia, G.C. and M.S. Baliga (2002). *Syzygium cumini* (Jamun) reduces the radiation-induced DNA damage in the cultured human peripheral blood lymphocytes: A preliminary study. *Toxicology Letters*, 132: 19-25.

Iftikhar, H., D. Ahmed and M.T. Qamar (2019). Study of phytochemicals of *Melilotus indicus* and alpha-amylase and lipase inhibitory activities of its methanolic extract and fractions in different solvents. *Chemistry Select*, 4: 7679-7685.

Lima, .LA., A.C. Siani, F.A. Brito, A.L.F. Sampaio, M.D.M.O. Henriques and C.A.D. Riehl (2007). Correlation of anti-inflammatory activity with phenolic content in the leaves of *Syzygium cumini* (L.) skeels (Myrtaceae). *Quimica Nova*, 30: 860-864.

Meragelman, T.L., K.D. Tucker, T.G. McCloud, J.H. Cardellina and R.H. Shoemaker (2005). Antifungal flavonoids from *Hildegardia barteri*. *Journal of Natural Products*, 68: 1790-1792.

Migliato, K.F. (2005). Standardization of the extract of. *Syzygium cumini* (L.) skeels fruits through the antimicrobial activity. *Caderno de Farmacia*, 21: 55-56.

Muthulakshmi, A., R.J. Margret and V.R. Mohan (2012). GC-MS analysis of bioactive components of *Feronia elephantum* Correa (Rutaceae). *Journal of Applied Pharmaceutical Science*, 2: 69-74.

Ozdemir, G, N.U Karabay, M.C. Dalay and B. Pazarbasi (2004). Antibacterial activity of volatile component and various extracts of *Spirulina platensis*. *Phytotherapy Research*, 18: 754-757.

Plathia, M., V.K. Wali, P. Bakshi and N. Sharma (2018). Studies on variability of seedling origin jamun (*Syzygium cumini* (L.) Skeels) germplasm growing in subtropical region of Jammu, India. *International Journal of Current Microbiology and Applied Sciences*, 7: 3363-3368.

Sherif, F.M. (2019). Pharmacognostical and biological evaluation of cultivated *Syzygium cumini* (L.) Skeels (Jambolan) in Libya. *EC Pharmacology and Toxicology*, 7: 721-734.

Shobier, A.H., S.A.A. Ghani and K.M. Barakat (2016). GC/MS spectroscopic approach and antifungal potential of bioactive extracts produced by marine macroalgae. *The Egyptian Journal of Aquatic Research*, 42: 289-299.

Singh, B., J.P. Singh, A. Kaur and N. Singh (2018). Insights into the phenolic compounds present in jambolan (*Syzygium cumini*) along with their health-promoting effects. *International Journal of Food Science and Technology*, 53: 2431-2447.

Singh, J.P., A. Kaur, N. Singh, L. Nim, K. Shevkani, H. Kaur and D.S. Arora (2016). In vitro antioxidant and antimicrobial properties of jambolan (*Syzygium cumini*) fruit polyphenols. *LWT-Food Science and Technology*, 65: 1025-1030.

Sosa, A.A., S.H. Bagi and I.H. Hameed (2016). Analysis of bioactive chemical compounds of *Euphorbia lathyrus* using gas chromatography-mass spectrometry and fouriertransform infrared spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research*, 8: 109-126.

Suryajayanti, M.F., A.L. Dewi, B.A. Nurrahma, A. Dewi, S. Putri, Z. Khairia and A. Farmawati (2017). Effect of ethanol extract of *Syzygium cumini* (L.) pulp on blood pressure and malondialdehyde level of chronic restraint stress rats. *Journal of Medical Sciences*, 49: 114-120.

Vaithiyanathan, V. and S. Mirunalini (2015). Quantitative variation of bioactive phyto compounds in ethyl acetate and methanol extracts of *Pergularia daemia* (Forsk.) Chiov. *Journal of Biomedical Research*, 29: 169-172.

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