REVIEW ARTICLE

Analyses of phytochemical constituents in *Justicia gendarussa* extract: A mini review

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Abstract:

Justicia gendarussa is a plant from the Acanthaceae family with numerous medicinal benefits. Several studies have reported the phytochemical compounds of *J. gendarussa* extract however, the method used for detection of the chemical constituents have not been reviewed. Hence, the purpose of this review is to look at various methods used in the detection of chemical constituents of the *J. gendarussa* extract. Previous research were collected from Google Scholar which resulted in thirty-seven articles related to *J. gendarussa* and detection of its phytochemical constituents. From the review, detection methods involved were qualitative screening, thin layer chromatography, liquid chromatography, and gas chromatography. While phytochemical compounds detected were alkaloid, anthraquinons, betacyanin, linoleic acid, oleic acid, apigenin, kaempferol, naringenin, vitexin, Gendarusin A, glycosides, anthocyanin, tannins, carotenoids, saponins, steroids and carbohydrates. Results of analysis demonstrated that every method has its advantages and disadvantages. Therefore, this review will help researchers in choosing the best and most suitable method for phytochemical analysis depending on their need and interest.

Keywords: detection, *Justicia gendarussa*, phytochemicals

1. INTRODUCTION

Justicia gendarussa is a medicinal plant from the Acanthaceae family. It is a moist, fast growing, and evergreen plant found mainly in humid areas (Nirmalraj & Perinbam, 2015). Hence it is mostly found in Indonesia, Africa, Brazil, and Central America (Ramees et al., 2019). However, it also found in India, China, Malaysia, Sri Lanka, the Philippines, and Bangladesh (Ningsih et al., 2015). Since it can be found in many places, this plant is called by many different names according to different regions such as in Malay it is called 'Daun Rusa' (Khare, 2007), 'Black adusa' in English (Saha et al., 2012) and in Myanmar it is commonly known as pha-wa-net (Aye et al., 2019). In Tamil it is called as 'Karunocchi', 'Kala bashimb' in Hindi, and 'Bhutakeshi' in Sanskrit (Pal & Rahaman, 2015).

This plant is a branched undershrub and 0.8 to 1.5 m high with long leaves. The leaves are flat, lanceolate or linear lanceolate, light green underneath and dark green on top. The roots and the stems are dark violet. Flowers from the uppermost axils of the leaf are 5-12,5 cm long; white colored with purple spots and clustered in interrupted spikes (Vijayakumar et al., 2019; Raghu & Agrawal, 2016).

According to the World Health Organization (WHO), nearly 80% of the world's population utilize plant extracts and its constituent as medication in traditional therapies (Neena et al., 2019). Similarly, *J. gendarussa* have been traditionally used as remedies for various diseases. In traditional Indian and Chinese medicine, the plant's leaf is prescribed for the treatment of diseases such as fever, hemiplegia, rheumatism, arthritis, headache, earache, muscle pain, respiratory disorders and digestive disorders (Nirmalraj & Perinbam, 2015). In the Papuan tribe in Indonesia, *J. gendarussa* is used traditionally for male contraception by decreasing the concentration of testosterone and the dispersibility of cumulus oophorus *in vitro* (Mnatsakanyan et al., 2018). Therefore, it could reduce spermatozoa hyaluronidase (Matsunami, 2018). In addition, it has also been used in the treatment of bronchitis, eye infections, vaginal discharges, dysentery, eczema and jaundice (Venkatachalam et al., 2019). As for scientific studies, various parts of the plant have been explored such as their leaves and roots for their various phytochemical, antibacterial and antioxidant properties (Kumar et al., 2017).

Phytochemical analyses are very useful in determining the chemical components of medicinal plants which will give insights on their potential health benefits. The phytochemical screening carried out on *J. gendarussa* leaves extract indicated the existence of several active compounds that play vital roles in fighting diseases. Some of the compounds reported by previous studies were carbohydrates, glycosides, alkaloids, flavonoids, tannins, saponins, phenolic

compounds, steroids and triterpenoids (Prasad et al., 2017; Vijayakumar et al., 2019). To detect the phytochemicals present in the extract there are various methods of detection which can be used including the qualitative and quantitative analysis. Qualitative analysis is used to determine the presence of compounds such as alkaloids, tannins, amino acids, flavonoids, phenols, carbohydrates, proteins, saponins and glycosides via visible chemical reactions. There are also other methods that can be used to analyse them both qualitatively and quantitatively at once. Examples are thin layer chromatography, gas chromatography and liquid chromatography methods. However, until today, no review on the methods used to determine the *J. gendarussa* constituents has been published. Thus, this study aims to review the various methods used in detection of chemical constituents of *J. gendarussa*. Based on previous research, methods used in the detection of chemical constituents as well as the various phytochemical constituents found in *J. gendarussa* were summarized.

2. RESULTS AND DISCUSSION

2.1 Analyses of *J. gendarussa* extract

2.1.1 Qualitative Screening

Qualitative phytochemical screening is the first step to determine the existence of chemical compounds in the *J. gendarussa* extract. Most researchers began their study by conducting the preliminary phytochemical analysis which qualitatively examine the plant extract before proceeding to quantitative analysis. This method allows researcher to detect the presence or absence of various types of phytochemical constituents. In general, qualitative analysis involves chemical tests using reagents against the *J. gendarussa* extract. Resulting chemical reaction will indicate the existence of the compound of interest. Results of the tests will be reported as presence or absence of the chemical compound in the plant extract. Table 1 demonstrates a few examples of the tests.

Based on the qualitative screening analysis done by previous researchers, several chemical constituents were evidently found in the *J. gendarussa* extract. The chemical constituents that have been found through this method were carbohydrates (Ramees et al., 2019; Vijayakumar et al., 2019; Kumar et al., 2017), glycosides (Nirmalraj & Perinbam, 2015; Kumar et al., 2017; Vijayakumar et al., 2019), alkaloids (Nirmalraj & Perinbam, 2015; Kumar et al., 2017; Ramees et al., 2019; Vijayakumar et al., 2019), flavonoids (Nirmalraj & Perinbam, 2015; Kumar et al., 2017; Ramees et al., 2019; Nongmaithem, 2019; Vijayakumar et al., 2019), tannins (Nirmalraj & Perinbam, 2015; Kumar et al., 2017; Ramees et al., 2019; Vijayakumar et al., 2019) and saponins (Nirmalraj & Perinbam, 2015; Kumar et al., 2017; Ramees et al., 2019; Vijayakumar et al., 2019). Furthermore, phenols (Nirmalraj & Perinbam, 2015; Ramees et al., 2019), steroids (Vijayakumar et al., 2019), anthraquinons (Nirmalraj & Perinbam, 2015), terpenoids (Nirmalraj & Perinbam, 2015), protein (Kumar et al., 2017), betacyanin (Kumar et al., 2017) and anthrocyanin (Kumar et al., 2017) have also been found in the plant extract.

Table 1. Procedures for phytochemical screening and indications for presence of compound.

Test	Procedure	Indication for positive result
Alkaloids (Mayer's test)	2mL of 2N hydrochloric acid (HCl) and Mayer's reagent (Potassium mercuric iodide solution) are mixed together with 1 mL of crude extracts.	Formation of a turbid white creamy precipitate.
Flavonoids	In 1mL of crude extracts, a few drops of 1% Ammonia (NH_3) are added.	An intense yellow colour.
Glycosides	1mL of crude extract and 1mL of concentrated sulphuric acid are mixed together. Fehling's solution is added to the solution.	A black-red precipitate
Tannins	1mL of crude extract is pipetted with 2mL of % $FeCl_3$.	A blue-black precipitate.
Terpenoids	In 0.5mL of acetic anhydride, 1mL of crude extract is dissolved. The mixture is added with few drops of concentrated sulphuric acid.	A bluish green precipitate.
Saponins	5ml of crude extract is mixed together with 5ml of deionized distilled water in a falcon tube. Then, the mixture is shaken vigorously.	Presence of foam that last for 15 minutes.

2.1.2 Thin Layer Chromatography

Thin layer chromatography is one of the separation methods used in the detection of chemical constituents of *J. gendarussa* extract. In this method, a piece of glass, metal or rigid plastic is used and coated with a thin layer of silica gel as the stationary phase (Altemimi et al., 2017). Silica gel was covered all over the surface of the plate. The effectiveness along with the nature of separation of the plate can be increased by lowering the thickness of the silica layer (Banu & Cathrine, 2015). In terms of *J. gendarussa*, the thickness

of the thin layer of silica gel that have been applied by previous researchers were 0.25 mm (Patel & Zaveri, 2014; Widodo et al., 2018; Neena et al., 2019).

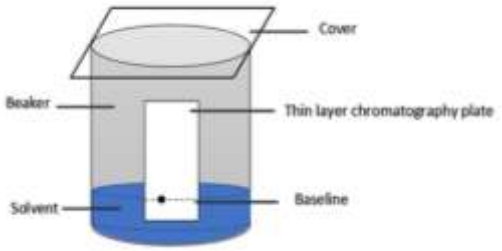

Figure 1. Simple schematic diagram of Thin Layer Chromatography

In this method, solvent is used as the mobile phase and is put together into the glass chamber as demonstrated in Figure 1. Using different ratio of solvents will elute the sample easier and give a better separation in the mobile phase. In the preparation of *J. gendarussa* extract, researchers used different kinds of solvents at various ratios. For example, the study by Neena et al. (2019) used petroleum ether and acetone in a ratio of 1:1. On the other hand, Widiyanti et al., (2018) used chloroform and methanol in a 9:1 ratio while Patel and Zaveri (2014) prepared alcoholic concentrate of leaf using n-butanol: formic corrosive (4.5:0.5). Some studies even used more than two types of solvents such as the usage of butanol: glacial acetic acid: water (4:1:5) by Widiyanti et al. (2018) to determine the alkaloid and flavonoid compounds of *J. gendarussa* leaves. In 2014, Patel and Zaveri used n-butanol: methanol: water: formic corrosive in a 2.5:1:1:0.5 ratio as the mobile phase in their study.

Solvent and the silica coated plate will then be put in a chamber as the elution process needs to be done in a closed system such as in a beaker or a glass chamber. The most desirable saturation condition is at relative dampness of 60% ±5 for 30 minutes at room temperature (Patel & Zaveri, 2014). In the chamber, the solvent will tend to move upwards towards the end of the plate by capillary action (Kumar et al., 2013). Different chemical constituents of the plant extract will stop at different locations based on the polarity of the components in the extract. Observation of the separated chemical constituents can be done using ultraviolet (UV) lamp or chemical stains for the non-active UV compounds. To aid the observation process of the chemical component present in the *J. gendarussa* extract, Widodo et al. (2018) have used Dragendorf reagent for alkaloid detection and borate citric for flavonoid detection. As for Patel and Zaveri (2014), separated chemical constituents of *J. gendarussa* extract were seen under UV light at 254 nanometer (nm) and 366 nm. Once detected, the spots can be used in the determination of the polarity and qualitative description of the molecules by calculating the ratio distance travelled by the compound and the solvent known as retention factor (Rf value) (Patel & Zaveri, 2014).

Some of the advantages from using this method are it is a simple procedure to set up and the cost involved is not expensive. Hence it is suitable for studies with low budget (Altemimi et al., 2017). This method also only takes about 30-60 minutes to run for qualitative analysis of any compounds (Kumar et al., 2013). However, the thin layer chromatography method is only applicable to non-volatile compound and it is not a fully automated procedure hence it might lead to misinterpretation of the results.

Various research has been carried out using thin layer chromatography method in detection of phytochemical constituents of *J. gendarussa* extract. Study by Patel and Zaveri (2014) has found several compounds such as phenolic, carbohydrate, flavonoid, steroid, carotenoid, alkaloid, and triterpenoid from the extract. Another study by Widodo et al., (2019) had detected compounds such as alkaloid, flavonoid, phenolic, saponin and steroid. However, some studies only used this method to specifically detect alkaloid and flavonoid in the *J. gendarussa* extract (Widiyanti et al., 2018; Widodo et al., 2018 Neena et al., 2019).

2.1.3 Liquid Chromatography

Liquid chromatography is another method usually used in the separation of phytochemical constituents of plant extract. This method still adheres to the same principle as other chromatography method which is the involvement of stationary and mobile phases. However, in this method, sample is injected into the mobile phase and then passes through the column as illustrated in Figure 2. Example of column used is column made of silica (2.2 µm, 120 Å, 2.1 x 100 mm) which was used in the study conducted by Ningsih et al., (2015) and Ratih et al., (2019) to identify the *J. gendarussa* chemical compounds.

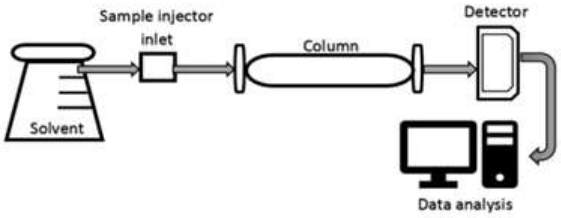

Figure 2. Simple schematic diagram of Liquid chromatography system

In the liquid mobile phase, sample will flow through the column by gravitational force and become separated. Non-polar or less polar solvent is preferable when choosing the solvent to aid the separation process. Non-polar compound will flow down the column faster than polar compound because polar compounds have the tendency to become attached to the column surface longer. In 2019, study by

Ratih et al. has used a mixture of ammonium acetate and methanol as their mobile phase to identify phytochemical constituents of *J. gendarussa* extract. Mobile phase used by Ningsih et al., (2015) also consisted of ammonium acetate and methanol. Results of the separation process will be interpreted by a detector connected at the exit of the column. One of the detectors which is commonly used is mass spectrometer. It can be used to identify and quantify the compounds based on their molecular weight (Ningsih et al., 2015; Ratih et al., 2019).

On the other hand, high performance liquid chromatography (HPLC) is an upgraded version of the liquid chromatography method (Pang et al., 2016). The HPLC method still maintains the stationary phase and mobile phase such as that of the liquid chromatography technique. However, the procedures involved in inserting sample and solvent into the column is slightly different than that of liquid chromatography method. To introduce solvent-sample mixture into the column, it uses a high-pressure pump with a constant flow rate as shown in Figure 3 (Feng et al., 2019). The usual optimal constant rate used by previous researchers was 1mL/min (Raghu & Agrawal, 2016; Mnatsakanyan et al., 2018). This is to aid the process of separation of compounds before they flow out of the column. To reach the detector, the speed of every compound depends on the it's nature and the mobile phase used. Once the separation process ends, the compounds will come out of the column and flow to the connected detector. Previous study by Mnatsakanyan et al., (2018) utilized a column with 250 mm of length, 4.6 mm of internal diameter and a solvent system which consisted of methanol and water in their research on quantitative evaluation of male contraceptive property of *J. gendarussa* extract and identification of a new amino benzyl derivative. However, Raghu and Agrawal, (2016) used a column with the exact same size but used a mixture of acetonitrile and 0.1% orthophosphoric acid instead for the mobile phase.

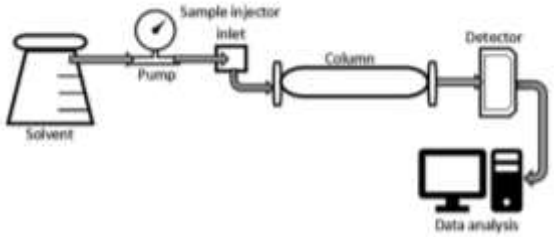

Figure 3. Simple schematic diagram of High-performance liquid chromatography system

To detect the resulting compound, Raghu and Agrawal (2016) used the UV detector for the detection of vitexin in the *J. gendarussa* extract. Detection was conducted using the intensity of the UV light at wavelength 335 nm. Another type of detector which had been used to determine the phytochemical constituents in the plant extract especially for non-volatile compounds, is evaporative light scattering detector (ELSD). ELSD was used under the parameters of 45°C temperature and pressure of 3.5 bar for the profiling and quantification of the compounds found in *J. gendarussa* extract (Mnatsakanyan et al., 2018).

Each method has its own benefits and limitations. Liquid chromatography method, prior to being upgraded into HPLC method, was an efficient method to separate compounds at low temperature. However, its' disadvantage was it is time consuming. This is because in liquid chromatography method, the flow rate of solvent fully depends on the gravity during the elution process (Thammana, 2016). In the following years, HPLC was introduced and has become the choice of many researchers due to its privileges. For instance, the amount of sample and solvent required in the procedure were reduced and it produced results with high sensitivity (Zhu & Chen, 2017). HPLC was also less time consuming with high rate of sensitivity (Vare et al., 2019). This method is also suitable for analysis of compounds that are either insoluble or poorly soluble in water (Juszczak et al., 2019). Nonetheless, HPLC method is a quite complicated procedure and requires a skilled and knowledgeable person to operate the system (Zhu & Chen, 2017) and at the same time it is costly (Vare et al., 2019).

Liquid chromatography mass spectrometer analyses by Ratih et al., (2019) have revealed the presence of thirty-five metabolites in the *J. gendarussa* extract. They found that the major constituents were alkaloids, fatty acids and apigenin glycosides. Their findings were more extensive compared to that of Ningsih et al., (2015) in which only six compounds were proposed from their study. There were justidrusamide A or justidrusamide B, 6-desmethylprazosin or 7-desmethylprazosin, 1,5-dideoxy-3-C-[({5-hydroxy-2-[(5-oxooxolane-2-carbonyl)-amino]phenyl}methoxy)carbonyl] pentitol,6,8-di-C-α-L-arabinosyl-apigenin,4-[(morpholin-4yl)(oxo)acetyl]phenyl hexopyranoside, and justidrusamide C or justidrusamide D. In 2016, Raghu and Agrawal had reported, in accordance with their main objective which was to identify vitexin in the leaves extracts of *J. gendarussa*, the identification of vitexin by using the HPLC method. By using ELSD, Mnatsakanyan et al., (2018) found three main constituent peaks in the methanol extract. The three main peaks were gendarusin A, justidrusamide A, and justidrusamide B. Other minor constituents were justidrusamide C, justidrusamide D, and justidrusamide E.

2.1.4 Gas Chromatography

Another method which has been used to separate different types of compounds in *J. gendarussa* extract is gas chromatography. Gas chromatography is a separation technique using a column as the stationary phase. The column also functions as the holder for the sample. The main difference between liquid chromatography and gas chromatography is the mobile phase used. In the liquid chromatography method, a solvent is used as its mobile

phase, while inert gas is used in the gas chromatography method (Hadi & Hameed, 2017). The most common carrier gases used by researchers was helium or nitrogen (Coskun, 2016). There have been a few studies which utilized helium as their mobile phase with the flow rate between 1.0 ml/min to 1.3 ml/min (Ayob et al., 2017; Wahyuni et al., 2017; Yadav et al., 2017). In addition, the inner wall of the capillary column is coated with liquid stationary phase to increase the sensitivity of the separation process (Rahman et al., 2015). Figure 4 demonstrates the schematic diagram of a gas chromatography apparatus.

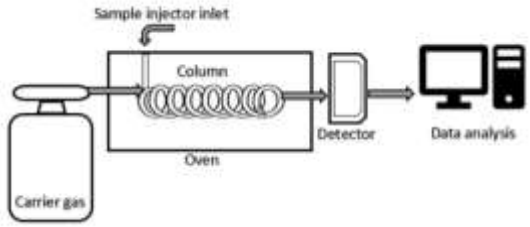

Figure 4. Simple schematic diagram of Gas chromatography system

In previous studies involving the use of gas chromatography for the detection of phytochemicals within the *J. gendarussa*, several types of columns have been used. Yadav et al., (2017) had utilized a column with 30.0 m length, 0.25 mm width and film thickness of 0.25 µm. However, a fused silica capillary column (30.0 m × 0.32 mm, film thickness 0.25 µm) was used by Ayob et al., (2017) in their studies. Besides that, study by Wahyuni et al., (2017) used a capillary column coated with 5% phenylmethyl siloxane, 30.0 m length, 0.32 mm width and 0.2 um of film thickness to examine the phytocomponents of methanolic extracts of the *J. gendarussa*.

Similar to liquid chromatography, sample loaded into the column is in the liquid state. Study done by Murugesan (2017) had used three types of solvents including methanol, chloroform, and oil ether while a study by Ayob et al., (2017) used crude extract of *J. gendarussa* leaves. When the column is heated, the sample is instantly vaporised and become mixed with the carrier gas. The column's temperature will then be gradually increased and then maintained at a temperature that has been set throughout the process. This is to allow the elution process of low boiling and less volatile compounds at low temperature, while high boiling compounds at higher temperature. Study by Yadav et al., (2017) had used a temperature range of 60°C to 300°C with a rate of 10°C/min. In comparison, Wahyuni et al., (2017) used a slightly lower temperature which was 50°C-280°C with a rate of 100°C/min and Ayob et al., (2017) used 100°C-275°C with the rate of 10°C/min. Heating the column will allow the sample to become vaporized then be carried by the mobile phase. The rate of migration relies upon the number of chemical compounds scattered in liquid phase. The higher the level of compound in the gaseous form, the faster the migration process to the exit of the column (Banu & Cathrine, 2015). As shown in Figure 4, a detector is attached at the end of the column to record the time when the compounds reached the detector. The type of detector normally used by researchers in their study on *J. gendarussa* were mass spectrometer (Murugesan, 2017; Wahyuni et al., 2017; Yadav et al., 2017) and flame ionization detectors (Ayob et al., 2017).

Benefits of using gas chromatography for phytochemical analysis are its simplicity, sensitivity, and effectiveness (Hadi & Hameed, 2017). Aside from these advantages, it is useful for detection of volatile compounds. However, the drawback is that the gas chromatography method is ineffective for non-volatile compounds (Xu et al., 2017). Furthermore, the use of inert gases such as helium as the mobile phases in this method is hazardous and costly (Juszczak et al., 2019).

The use of gas chromatography method in the detection of chemical compounds in the *J. gendarussa* extract resulted in discovery of various compounds which have also been reported by previous studies. GC-FID (gas chromatography-flame ionization detector) was used to detect and quantify naringenin and kaempferol compounds contained in young and mature leaves in *J. gendarussa* (Ayob et al., 2017). The study by Yadav et al., (2017) has shown the presence of oleic acid, 9,12-octadecadienoic acid, 6,9,12-octadecatrienoic acid and estra-1, 3,5 (10)-trein-17-β-ol in the *J. gendarussa* extract. In addition, studies conducted by Murugesan (2017) and Wahyuni et al., (2017) have successfully detected 23 and 26 compounds, respectively.

2.2 Phytochemical constituents of *J. gendarussa* extract

Based on the studies reviewed, there were many chemical constituents found in *J. gendarussa* extracts by using various methods of detection. Each chemical constituent has their own benefit and medicinal usage. The important chemical constituents detected in the *J. gendarussa* extracts and their medicinal usage for human are as listed in Table 2.

Table 2. Important chemical constituents detected in *Justicia gendarussa* extracts and their medicinal usage for human.

Chemical constituents	Medicinal usage	References
Alkaloid	Anti-inflammatory, Anticancer, Antimicrobial, Antifungal, Analgesic, Anesthetic, Neuropharmacologic, Pain reliever.	(Patel & Zaveri, 2014; Nirmalraj & Perinbam, 2015; Kumar et al., 2017; Widiyanti et al., 2018; Ramees et al., 2019; Neena et al., 2019; Ratih et al., 2019; Vijayakumar et al., 2019; Widodo et al., 2019)

Anthraquinons	Antibacterial, Anti-fungal, Antiviral, Antioxidant, Constipation reliever.	(Nirmalraj & Perinbam, 2015)
Betacyanin	Antioxidant, Anticancer.	(Kumar et al., 2017)
Fatty acid (Linoleic acid)	Anti-inflammation, Anticarcinogenic, Anti-arthritic, Antieczemic, Antiacne, Nematicide, Insectifuge.	(Yadav et al., 2017)
Fatty acid (Oleic acid)	Induce apoptosis in carcinoma cells.	(Yadav et al., 2017)
Flavonoid (Apigenin)	Antidepressant, Antitumor, Anti-inflammatory, Antioxidant.	(Ratih et al., 2019)
Flavonoid (Kaempferol)	Inhibit cell proliferation, antioxidant, Prevent arteriosclerosis	(Ayob et al., 2017)
Flavonoid (Naringenin)	Antiproliferative, Antibacterial, Anticarcinogenic, Cholesterol lowering agent, Help fight retinal disease linked to diabetes.	(Ayob et al., 2017)
Flavonoid (Vitexin)	Prevent heart disease	(Raghu & Agrawal, 2016)
Flavonoid (Gendarusin A)	Male contraceptive properties, anti-HIV.	(Mnatsakanyan et al., 2018)
Glycosides	Antioxidant, Antitumor, Anticancer, Anti-diabetes, Hepaprotective.	(Nirmalraj & Perinbam, 2015; Kumar et al., 2017; Ramees et al., 2019; Ratih et al., 2019; Vijayakumar et al., 2019)
Phenols (Anthocyanin)	Anti-allergic, Anti-inflammation, lowering blood pressure and reduce tumor growth.	(Kumar et al., 2017)

3. CONCLUSION

The existence of chemical constituents of the plant can be determined by carrying out analyses using standard procedures. As for the detection and quantification of phytochemical compounds in the *J. gendarussa* extract, previous researchers have used various types of methods. Qualitative screening is the preliminary screening step in the detection of chemical compounds in the *J. gendarussa* extract. As for the detection and quantification of phytochemical compounds in the extract, previous researchers have used various types of chromatography methods.

Chromatography is a method used to separate compounds in a mixture that involves a mobile phase and a stationary phase. The chromatographic techniques which have been used by several groups working on *J. gendarussa* extracts were thin layer chromatography, liquid chromatography, and gas chromatography. Thin layer chromatography is the simplest procedure, but it is not fully automated. HPLC is less time consuming compared to liquid chromatography but in terms of efficiency, gas chromatography is more effective and produced more precise result than HPLC when used to quantify chemical constituents in plant extract. Gas chromatography also has its drawback in which the compounds being analysed must be volatile and thermally stable.

Many chemical constituents were found in the *J. gendarussa* extract by the mentioned techniques. The chemical constituents found were phenols, kaempferol, naringenin, tannins, anthocyanins, vitexin, carotenoids, saponins, apigenin, anthraquinons, glycosides, betacyanin, amino acids, and fatty acid.

Over the years, many studies have been done on *J. gendarussa*. However further identification of the phytochemicals present in the extract should be carried out using other methods of detection. Another aspect that should be given attention to is quantification of the chemical constituents in the plant extract.

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REFERENCES

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, 6(4). https://doi.org/10.3390/plants6040042

Aye, M. M., Aung, H. T., Sein, M. M., & Armijos, C. (2019). A

review on the phytochemistry, medicinal properties and pharmacological activities of 15 selected Myanmar medicinal plants. *Molecules*, *24*(2). https://doi.org/10.3390/molecules24020293

Ayob, Z., Jamil, S., Mohd Bohari, S. P., Ahmad, F., & Abd Samad, A. (2017). Detection of naringenin and kaempferol in Justicia gendarussa leaf extracts by GC-FID. *Sains Malaysiana*, *46(3*), 457–461. https://doi.org/10.17576/jsm-2017-4603-13

Banu, K. S., & Cathrine, L. (2015). General Techniques Involved in Phytochemical Analysis. *International Journal of Advanced Research in Chemical Science*, *2(4*), 25–32. www.arcjournals.org

Coskun, O. (2016). Separation Tecniques: CHROMATOGRAPHY. *Northern Clinics of Istanbul*, *3*(2), 156–160. https://doi.org/10.14744/nci.2016.32757

De Silva, G. O., Abeysundara, A. T., & Aponso, M. M. W. (2017). Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. *American Journal of Essential Oils and Natural Products*, *5*(2), 29–32.

Feng, W., Li, M., Zhiyou, H. & Zhang, J. (2019). Analytical Methods of Isolation and Identification. 10.5772/intechopen.88122.

Hadi, M. Y., & Hameed, I. H. (2017). Uses of Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Analysis of Bioactive Chemical Compounds of Lepidium sativum : A Review. *Research Journal of Pharmacy and Technology*, *10*(11), 4039. https://doi.org/10.5958/0974-360x.2017.00732.6

Juszczak, A. M., Zovko-Končić, M., & Tomczyk, M. (2019). Recent trends in the application of chromatographic techniques in the analysis of luteolin and its derivatives. In *Biomolecules* (Vol. 9, Issue 11). https://doi.org/10.3390/biom9110731

Khare, C. P. (2007). Justicia gendarussa Burm. f. *Indian Medicinal Plants*, *47*, 1–1. https://doi.org/10.1007/978-0-387-70638-2_844

Kumar, D., Mary, D. J., & Kumari, M. (2017). Screening of medicinal plant Justicia gendarussa Burm. f for its antibacterial and antioxidant activity from different localities. *International Journal of Current Research*, *9*(4), 49063–49066.

Kumar, S., Jyotirmayee, K., & Sarangi, M. (2013). Thin layer chromatography: A tool of biotechnology for isolation of bioactive compounds from medicinal plants. *International Journal of Pharmaceutical Sciences Review and Research*, *18*(1), 126–132.

Matsunami, K. (2018). Natural products chemistry of global tropical and subtropical plants. *Chemical and Pharmaceutical Bulletin*, *66*(5), 467–468. https://doi.org/10.1248/cpb.c18-ctf6605

Mnatsakanyan, M., Queiroz, E., Marcourt, L., Prajogo, B., & Wolfender, J.-L. (2018). Quantitative Evaluation of Various Preparations and Extracts of the Male Contraceptive Justicia gendarussa and Identification of a New Aminobenzyl Derivative. *Planta Medica International Open*, *5*(01), e30–e38. https://doi.org/10.1055/a-0584-0321

Mukthi, T. (2016), A Review on High Performance Liquid Chromatography (HPLC) by Research & Reviews: Journal of Pharmaceutical Analysis, e-ISSN: 2320-0812, p-ISSN: 2347-2340, July – September; 5(2).

Murugesan, S. (2017). Phytochemical Screening, GC-MS Analysis of Bioactive Compounds and Antibacterial activity from Justicia gendarussa Burm.F. Stem. *International Journal of Pharmacognosy and Phytochemical Research*, *11*(1), 23–28.

Neena, A., P, M. M., & Thomas, B. (2019). Phytochemical and Antimicrobial evaluations some selected plants in family Acanthaceae. *Journal of Pharmaceutical Advanced Research*, *2*(2), 479–483.

Ningsih, I. Y., Purwanti, D. I., Wongso, S., Prajogo, B. E. W., & Indrayanto, G. (2015). Metabolite profiling of Justicia gendarussa Burm. F. leaves using UPLC-UHR-QTOF-MS. *Scientia Pharmaceutica*, *83*(3), 489–500. https://doi.org/10.3797/scipharm.1411-08

Nirmalraj, S., & Perinbam, K. (2015). Studies on phytochemical screening and in vitro antioxidant activity of ethyl acetate leaf extract of Justicia gendarussa Burm. F. *Research Journal of Botany*, *10*(1), 30–36. https://doi.org/10.3923/rjb.2015.30.36

Nongmaithem, R. (2019). *Phytochemical study of selected medicinal plants used by the maring tribe of Chandel district ,*. *8*(4), 2155–2160.

Pal, K., & Rahaman, C. H. (2015). Phytochemical and Antioxidant Studies of Justicia Gendarussa Burm. F. an Ethnomedicinal Plant. *International Journal of Pharmaceutical Sciences and Research*, *6*(8), 3454. https://doi.org/10.13040/IJPSR.0975-8232.6(8).3454-62

Pang, B., Zhu, Y., Lu, L., Gu, F., & Chen, H. (2016). The applications and features of liquid chromatography-mass spectrometry in the analysis of traditional Chinese medicine. *Evidence-Based Complementary and Alternative Medicine*, *2016*. https://doi.org/10.1155/2016/3837270

Patel, S., & Zaveri, M. (2014). Trypsin and Protein Denaturation Inhibitory Activity of Different Fractionation and Isolated Compound of Leaf and Root of Justicia Gendarussa. *International Journal of Pharmaceutical Sciences and Research*, *5*, 5564–5571. https://doi.org/10.13040/IJPSR.0975-8232.5(12).5564-71

Prasad, N., Singh, B., & Puri, D. (2017). Pharmacognostic screening studies of Justicia Gendarussa burm. Leaves found in dehradun district of Uttarakhand. *Asian Journal of Pharmaceutical and Clinical Research*, *10* (Special Issue September), 83–87. https://doi.org/10.22159/ajpcr.2017.v10s4.21342

Raghu, M. G., & Agrawal, P. (2016). RP-HPLC Method for Identification and Quantification of Vitexin from the Plant Justicia gendarussa. *Journal of Chemical and Pharmaceutical Sciences*, *9*(4), 2143–2148.

Ramees, M. K., Mamath, K., Nileena, F., Rosemery, K., Sinju, P. R., Nobin, C. J., & Suresh, J. (2019). Phytochemical screening of *Justicia gendarussa*. *International Journal of Pharmacognosy and Chinese Medicine*, 3(1), 1-6.

Rahman,M.M, El-Aty, A.M.A, Choi, J.H., Shin, S.C., & Shim, J.H. (2015). *Basic overview on gas chromatography columns*. In:Anderson, J.L., Berthod, A., Estevez, V.P., Stalcup, A.M. (eds). Analytical separation science, 1st edn. Wiley-VCH Verlag GmbH & Co, KGaA, Weinheim, 823-834.

Ratih, G. A. M., Imawati, M. F., Nugroho, R. R., Purwanti, D. I.,

Wongso, S., Prajogo, B., & Indrayanto, G. (2019). Phytochemicals of gandarusa (Justicia gendarussa) and its preparations. *Natural Product Communications*, *14*(6), 1–10. https://doi.org/10.1177/1934578X19851406

Saha, M. R., Debnath, P. C., Rahman, M. A., & Islam, M. A. U. (2012). Evaluation of in vitro anthelmintic activities of leaf and stem extracts of Justicia gendarussa. *Bangladesh Journal of Pharmacology*, *7(1)*, 50–53. https://doi.org/10.3329/bjp.v7i1.10558

Vare, R. S., M.M., S., M.S., G., S.J., B., & Y.G., D. (2019). A Review :Development and Validation of RP-HPLC Method for Quantitative Analysis of Pharmaceutical Dosage Form. *World Journal of Pharmaceutical Research*, *8*(6), 502–532. https://doi.org/10.20959/wjpr20196-14926

Venkatachalam, D., Rahman, A., Sunny, B., & Jacob, J. (2019). Studies on Pharmacognostical, Preliminary Phytochemistry of Stem of Justicia gendarussa Burn. *Journal of Advances in Pharmacy Practices*, *1*(1), 34–40.

Vijayakumar, A. S., Jeyaraj, M., Selvakumar, M., & Abirami, E. (2019). Pharmacological Activity of Silver Nanoparticles, Ethanolic Extract From Justicia Gendarussa (Burm) F Plant Leaves. *5*(462), 462–475. https://doi.org/10.26479/2019.0502.33

Wahyuni, D. K., Ansori, A. N. M., & Vidiyanti, F. (2017). GC-MS analysis of phytocomponents in methanolic extracts of leaf-derived callus of Justicia gendarussa Burm.f. *Bioscience Research*, *14*(3), 668–677.

Widiyanti, P., Prajogo, B., & Widodo, A. (2018). Effect of varying incubation periods on cytotoxicity and virucidal activities of justicia gendarussa burm.F. leaf extract on hiv-infected molt-4 cells. *African Journal of Infectious Diseases*, *12*(Special Issue 1), 133–139. https://doi.org/10.2101/Ajid.12v1S.20

Widodo, A., Khumaidi, A., & A. Lasongke, P. F. (2019). Toksisitas Ekstrak Etanol dan Ekstrak Air dari Daun Jotang Kuda (Synedrella nodiflora (L.) Gaertn.), Daun Gandarusa (Justicia Gendarussa Burm.F.), dan Daun Pulutan (Urena lobata L.) dengan Brine Shrimp Lethality Test. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal)*, *5*(2), 198–205. https://doi.org/10.22487/j24428744.2019.v5.i2.13935

Widodo, A., Widiyanti, P., Prajogo, B., & Sciences, N. (2018). *Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Tadulako University, Palu ,*. *12*, 36–43.

Xu, Y., Li, Y., Maffucci, K. G., Huang, L., & Zeng, R. (2017). Analytical methods of phytochemicals from the genus gentiana. *Molecules*, *22*(12). https://doi.org/10.3390/molecules22122080

Yadav, D., Reshi, M. S., Uthra, C., Shrivastava, S., Srivastava, N., Narayana, S. K. K., & Shukla, S. (2017). Botanical and chemical fingerprinting of medicinal roots of Justicia gendarussa burm f. *Pharmacognosy Research*, *9*(2), 208–214. https://doi.org/10.4103/0974-8490.204643

Zhu, B., & Chen, Y. Y. (2017). Development and Application of Liquid Chromatography in Life Sciences. *Journal of Chromatography & Separation Techniques*, *08*(02), 8–11. https://doi.org/10.4172/2157-7064.1000358

