INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 21, No. 6, p. 298-306, 2022

RESEARCH PAPER OPEN ACCESS

GC-MS analysis and antibacterial activity of *Sphagneticola trilobata* (L.) JF Pruski

AVP. Karthikeyan*, A. Ramesh

PG and Research Department of Botany, Government Arts College (Affiliated to Bharathidsasan University) Karur, Tamilnadu, India

Key words: *Sphagneticola trilobata*, GC-MS, Biological activity, Antibacterial activity

http://dx.doi.org/10.12692/ijb/21.6.298-306 Article published on December 07, 2022

Abstract

Sphagneticola trilobata (L.) J.F. belongs to family Asteraceae. The presence of phytochemical compounds in the leaves of *S. trilobata* was evaluated using the standard procedure in methanol, acetone, ethanol and water extracts. Methanolic leaf extract of *S. trilobata* were analyzed for the presence of different volatile compounds by Gas chromatography-Mass spectroscopy (GC-MS) technique. There are about 14 different phytochemical constituents were identified through GC-MS analysis. The methanolic extract from *S. trilobata* leaves with the concentrations of 50, 75, and 100 µM were assessed for their effects against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Vibrio cholera* and *Escherichia coli*. Both gram positive and negative bacteria produced their zone of inhibition at moderate level against *S. trilobata* methanolic leaf extracts.

* **Corresponding Author:** AVP. Karthikeyan ✉ avpkarthi1974@gmail.com

Introduction

Wedelia known officially by the scientific name, *Sphagneticola trilobata* (L.) J.F. Pruski, but still commonly referred by its former name *Wedelia trilobata* (L.) Hitchc is a member of the family Asteraceae (formerly Compositae). The genus *Wedelia*, named in honor of Georg Wolfgang Wedel (1645-1721), Professor of Botany at Jena, Germany, has about 70 species that they are growing in tropical and subtropical regions. *W. trilobata* is a very attractive plant because of its nearly constant and foliage blooming. It is a creeping, mat-forming perennial herb. *W. trilobata* is widely spread in many tropical and subtropical regions and considered as a serious weed due to its fast growth rates. *W. trilobata* is an attractive source of many secondary metabolites (Zhang *et al.*, 2004; Nie *et al.*, 2004; Dai *et al.*, 2016; Mardina *et al.,* 2020a).

Traditionally, it is utilized by Indians for the treatment of back pain, muscle cramp, rheumatism, stubborn wounds, sores, swelling and arthritic pain, fever and malaria (Meena *et al.,* 2011). More number pharmacological activities of *S. trilobata* offers have been reported such as antimicrobial, antioxidant, anti-inflammatory, wound healing, anthelmintic and anticancer (Balekar *et al.*, 2012; Shanmuganathan and Karthikeyan, 2016; Buddhakala and Talubmook, 2020; Widiyowati *et al.,* 2020; Teo *et al.,* 2021; Mardina *et al.,* 2020b). In the Caribbean and Central America the aerial parts of this plant are used in traditional medicine against bronchitis, colds, abdominal pains, dysmenorrheal, and even as a fertility enhancer. In the village medicine, it is employed to treat back pain, muscle cramps, rheumatism, stubborn wounds, sores and swellings, and arthritic painful joint (Coe and Anderson, 1996; Mardina *et al.,* 2020c; Husain and Kumar, 2017). Therefore, the present study, aimed at to identify the preliminary phytochemicals components, biological and antibacterial activity of *S. trilobata*.

Materials and methods

Sample collection

The plant of *S. trilobata* was collected from Jawadhu hills located at Thiruvannamalai District, Tamil Nadu, India. The samples were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water.

Preparation of extracts

The leaf was shadow dried and it was made as a powder using electrical blender. Dried leaves powder were subjected to the solvent like methanol, ethanol, acetone and water for the bioactive active components extraction. About 5g of leaf powder were immersed in various solvent and after an hour and then it was kept for shaker on 3 days. Then it was filtered by using Whatman No.1 filter paper (Muhammad Tayyab and Durre Shahwar, 2015; Mooza Al-Owaisi, *et al.*, 2014).This extract was used for the preliminary phytochemical (Harborne, 1998) and GC-MS analysis and to study the antibacterial activity.

Phytochemical analysis

For the phytochemical screening four different leaf extracts of *S. trilobata* samples were used, we follow the standard procedure given by Trease and Evans (1989) and Harborne (1998).

Test for Alkaloids (Wagner's reagent)

A fraction of extract was treated with 3-5drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml of water) and observed for the formation of reddish brown precipitate (or colouration) which indicates the presence of alkaloids (Table 1).

Test for Flavonoids (Alkaline reagent test)

2ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of Flavonoids (Table 1).

Test for Saponins (Foam Test)

Test solution was mixed with water and shaken and observed that the formation of froth, which should be stable for 15 minutes. This indicates the presence of Saponins (Table 1).

Test for Terpenoids (Salkowki's test)

1ml of chloroform was added to 2ml of each extract followed by a few drops of concentrated sulphuric acid a

reddish brown precipitate produced immediately indicated the presence of Terpenoids (Table 1).

Test for Steroids (Liebermann Burchard test)

Crude extract was mixed with few drops of acetic anhydride boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a browning at the junction of two layers. Formation of green coloration of the upper layer indicates the presence of Steroids (Table 1).

Test for Glycosides (Keller Killiani Test)

Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides (Table 1).

Test for Tannins (Braymer's test)

2ml of extract was treated with 10% alcoholic ferric chloride solution. Formation of blue or greenish colour solution shows the presence of Tannins (Table 1).

Test for Quinones

A Small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate or colouration (Table 1).

Test for reducing sugars (Benedict's test)

The extracts were treated with benedict's reagent and heated on a water bath. Formation of an orange red precipitate indicates the presence of reducing sugar (Table 1).

Test for phenols (Ferric chloride test)

To the 1ml of solvent extracts, 3ml of distilled H_2O was added. To this, a few drops of neutral 5% $FeCl_3$ solution was added. Formation of a dark green colour indicated the presence of phenolics (Table 1).

Identification of bioactive constituents by GC-MS

GC-MS analysis of the methanolic leaf extract of the plant was done using a Shimadzu (GCMS-QP2020) model. The oven temperature is maintained at 220°C at a rate of 6°C/min; the carrier gas with a waft price of one ml/min. The cut up sampling technique changed into used to inject the pattern inside the ratio of 1:10. The molecular weight, name, chemical structure and molecular system of the additives have been tabulated in Table 2 and Fig. 1.

Table 1. Preliminary phytochemical constituents present in various solvent of leaf extracts of the *S. trilobata*.

SN	Phytoconstituents	Methanol	Acetone	Ethanol	Water
1	Alkaloids	+++	++	++	+
2	Flavonoids	+++	+	++	+
3	Saponins	+++	-	++	-
4	Triterpenoids	++	+	+	+
5	Steroids	++	++	++	+
6	Glycosides	+	+	++	+
7	Tannins	++	+	++	+
8	Quinones	++	+	+	-
9	Reducing sugar	+	-	+	-
10	Phenol	++	++	++	+

Table 2. Phytochemical components identified in the methanol leaf extracts of *S. trilobata* through GC-MS.

SN	Retention time (min)	Compound Name	Peak area (%)	Molecular weight	Molecular formula
1	4.316	1-Dodecene	3.45	140.266	$C_{10}H_{20}$
2	7.471	1-Tetradecanol	14.41	214.393	$C_{14}H_{30}O$
3	9.357	2,4-Ditert-Butylphenyl	3.59	646.9	$C_{42}H_{63}O_3P$
4	10.799	Tetradecanol	10.33	214.393	$C_{14}H_{30}O$
5	13.943	1-Heptadecene	5.29	238.45	$C_{17}H_{34}$
6	14.577	Neophytadiene	8.86	278.5	$C_{20}H_{38}$
7	15.201	6-Octen-1-Oil	2.42	128.21	$C_8H_{16}O$
8	16.239	1,2-Benzenedicarboxyl	6.70	166.14	$C_8H_6O_4$
9	16.826	1-Heptadecene	3.88	238.45	$C_{17}H_{34}$
10	17.154	Phenanthrene	5.99	178.23	$C_{14}H_{10}$
11	26.475	Squalene	6.51	410.73	$C_{30}H_{50}$
12	28.349	Octadecamethyl-	3.94	667.4	$C_{18}H_{54}O_9Si_9$
13	29.396	Beta. -Amyrin	7.70	144.17	$C_{10}H_8O$
14	29.612	Cyclononasiloxane	16.92	414.90	$C_{18}H_{54}O_9Si_9$

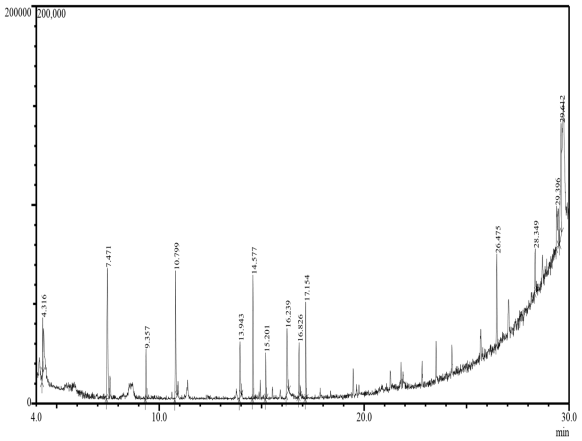

Fig. 1. GC-MS spectrum of methanolic leaf extracts of *S. trilobata*.

Antibacterial activity

To assess the antibacterial activities of S. *trilobata*, disc diffusion method was adopted given by Bauer *et al.,* (1966). Cultures of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Vibrio cholera and Escherichia coli* were used to lawn Muller Hinton agar plates evenly using a sterile swab.

Bacterial cultures are procured from MTTC, Chandigarh. The discs which had been impregnated with a series of plant extracts were placed on the Mueller Hinton agar surface. Each petridish contains one positive control, which is a standard commercial antibiotic disc (i.e. Ampicillin), one negative control (i.e. DMSO) and three treated discs. 50µM, 75µM and 100µM of concentration of the extract were introduced into the disc using sterile pipettes. Similarly, 100µM of amphicilin and DMSO was added in each disc separately. The plate was incubated at 37°C for 18 to 24 hours depending on the species of bacteria used in the test. After the incubation, the plates were examined for the inhibition zone. The inhibition zone was measured and tabulated in table 4.

Results and discussion

Phytochemical analysis

The presences of phytochemical compounds inside the leaves of *S. Trilobata* had been evaluated in methanol, acetone, ethanol and water extracts. Result obtained for qualitative screening of phytochemical leaf extracts of *S. Trilobata* are presented in table 1.

The consequences of methanol and ethanol leaf extracts confirmed that the presence of all 10 phytochemicals which includes steroids, flavonoids, triterpinoids, alkaloids, phenolic compounds, saponin, tannins, decreasing sugar and quinones. In acetone extract, presence of 8 out of 10 phytochemicals screened which include steroids, triterpinoids, alkaloids, tannins, phenolic compounds, sugars, quinones and saponins and there is no supply of lowering sugars and saponins, whereas in water extract lowering sugar, quinones and saponins are absent. A similar result was also observed by Misra *et al.,* 2011.

The phytochemical materials of this plant contribute to the whole antioxidant defense machine of the human body, and those secondary metabolites mixed with vitamins, combat free radicals, and for that reason they provide safety against dreadful diseases and early ageing. Flavonoids function a health promoting compound in antiinflammatory, oestrogenic, enzyme inhibition, antimicrobial, antiallergic, antioxidant, vascular and cytotoxic antitumour activities (Oliver-Bever, 1986; Block *et al.,* 1998; Harborne and Williams, 2000; Havsteen, 2002; Shanmugam *et al.,* 2011). Phenols were present in all extracts. Phenols have been found to be of great importance as they protect the human body from the oxidative stress, which cause many diseases, including cancer, cardiovascular and age related diseases (Robards *et al.,* 1999). Tannins have usual considerable attention in the field of nutrition, health and medicine, due to their antioxidant, antimicrobial and antiinflammatory properties (Santos-Buelga and Scalbert, 2000; Prasanth and Lakshmana, 2018; Sunita kanikaram *et al.,* 2018). Among the four solvent extracts, it was clear that the methanol solvent was most suitable for various phytochemical extractions present in *S. trilobata*. Thus, the phytochemicals present in *S. trilobata* indicates its ability as a source of principles that may supply novel medicine.

GC-MS Analysis

Methanolic extract of *S. trilobata* had been analyzed for the presence of various volatile compounds by means of fuel chromatography-Mass spectroscopy (GC-MS) technique. GC and MS walking time had been 40 minutes. The GC-MS chromatogram of the take a look at plant is provided in Fig.1. The energetic concepts with their retention time (RT), molecular method, molecular weight (MW) and top area are supplied in table 2. Retention indexes (RI) of the compounds have been determined by comparing their retention times of a chain and identification of each component changed into confirmed by using assessment of its retention index with statistics in the literature. The compound prediction is primarily based on Dr. Duke's Phytochemical and Ethnobotanical Databases by way of Dr. Jim Duke of the agricultural studies carrier/USDA.

Interpretation of Mass-Spectrum become done by means of the use of the database of countrywide institute trendy and era (NIST) having more than 62,000 patterns. The spectrum of the unknown additives became as compared with the spectrum of recognized components which was saved within the NIST library. There are about 14 unique phytochemical materials had been diagnosed via GC-MS analysis. The most prevailing major compounds of methanol extracts were 1-Tetradecanol (14.41 %), Tetradecanol (10.33 %), 2,4-Ditert-Butylphenyl (3.59 %), 1-Heptadecene (5.29%), Neophytadiene (8.86%),6-Octen-1-Oil (2.42%), 1-Heptadecene (3.88%), Phenantherne (5.99%), Squalene (6.51%), Octadecamethyl-(3.94%), Beta-Amyrin (7.70%), Cyclononasiloxane (16.92%). Many other compounds also detected in minor amounts in the leaf sample and the volatile oil were isolated by hydro distillation method from aerial parts of *S. trilobata* by Li *et al*., 2012 and Verma *et al.,* 2014. However, there may be variation in the chemical composition based on topography. The biological activities of the phytochemical components are listed in Table 3.

Table 3. Biological activities of the identified compound present in the methanol leaf extracts of *S. trilobata*.

SN	Retention time (min)	Compound Name	Nature of the compound	Biological activity
1	4.316	1-Dodecene	Alkane	Antibacterial, Antifungal
2	7.471	1-Tetradecanol	Saturated fatty alcohol	Antibacterial, Anti-inflammatory
3	9.357	2,4-Ditert-Butylphenyl	Phenol	Antioxidative, anticancer
4	10.799	Tetradecanol	Myristic acid common saturated fatty acids.	Antioxidant, antifungal activity. Correlated with higher cholesterol level
5	13.943	1-Heptadecene	Alkene	Antimicrobial, antioxidant
6	14.577	Neophytadiene	Terpenes	Antifungal, antioxidant, antipyretic, anti-inflammatory, analgesic and antimicrobial. Steroids, essential oils and presence of vitamin A.
7	15.201	6-Octen-1-Oil	Natural acyclic mono terpenoid	Used in perfumes, insect repellent and mite attractant, raw material for production of rose oxide, antibacterial, antifungal
8	16.239	1,2-Benzenedicarboxyl	Plasticizer compound	Antimicrobial, antifouling
9	16.826	1-Heptadecene	Alkene	Antimicrobial, antioxidant
10	17.154	Phenanthrene	Aromatic hydrocarbon	Cytotoxity, antimicrobial, spasmolytic, anti-inflammatory, anti-platelet aggregation, anti-allergic , anticancer and phyto toxicity
11	26.475	Squalene	Triterpene	Antioxidative, antibacterial, pesticide, antitumor, anti-inflammatory, chemoprotective stimulate immune system, antiaging.
12	28.349	Octadeca methyl-	Octodeca methyl cyclononasiloxane	Antioxidant, antibacterial
13	29.396	β -Amyrin	Triterpenoids	Antioxidant, antimicrobial
14	29.612	Cyclononasiloxane	Octodeca methyl cyclononasiloxane	Antioxidant, antibacterial

Anti-bacterial activity

The methanolic leaf extract of *S. trilobata* was screened for antibacterial activity by disc diffusion method against *Staphylococcus aureus, Staphylococcus epidermidis, Vibrio cholera and Escherichia coli* was presented in Table 4. The zone of inhibition of *Staphylococcus aureus* against 50µM, 75µM and 100µM of methanolic leaf extract of *S. trilobata* were showed in Table 4. The maximum zone of inhibition was produced 10.73±0.24 mm and it was observed in 100µM and the least zone of inhibition was observed 25µM that is 9.80±0.26 mm. Similarly, the maximum zone inhibition produced by *Staphylococcus epidermidis* was 13.23±1.02 mm and it was observed in 75µM and the least zone of inhibition was observed in 25µM that is 11.53±0.14 mm. While in *Vibrio cholerae* the maximum zone of inhibition was produced 12.13±0.21 mm and it was observed in 75µM and the least zone of inhibition was observed 25µM that is 10.83±0.23 mm.

The maximum zone of inhibition was produced by *Escherichia coli* was 16.46±0.34 mm and it was observed in 100µM and the least zone of inhibition was observed 25µM that is 11.83±0.23 mm. The methanolic leaf extract of *S. trilobata* inhibited the growth of all tested pathogenic bacteria. Large zone of inhibition of about 16.46 mm was observed against *E. coli*. A similar result was also observed by Chethan *et al*., 2012 in methanolic leaf extract of *S. trilobata*. The leaves extracts depicted the best potentiating effect at 100 µM with inhibitory zones of 16.46 and 11.83 mm for *E. coli* and *S. epidermidis*, respectively (Table 4). These results were close to inhibition zones of commercial antibiotic (ampicillin) at the concentration of 100 µM. The activity against *E. coli* and *S. epidermidis* may be caused by the presence of bioactive compounds, which can be enhanced through purification (Taddei and Rosas-Romero, 1999; Govindappa *et al.,* 2011a,b; Toppo *et al*., 2013; Wendels and Avérous, 2021).

Table 4. Zone of inhibition of *S. trilobata* methanolic leaf extracts against different microorganisms.

Bacteria	Zone of inhibition (mm)			Positive (control)	Negative (control)
	50 µM	75 µM	100 µM		
Staphylococcus aureus	9.80±0.26^{b}	10.03±0.18^{ab}	10.73±0.24^{a}	------	------
Staphylococcus epidermidis	11.53±0.14^{a}	13.26±1.02^{a}	11.83±0.37^{a}	10.72±0.46^{a}	------
Vibrio cholerae	10.76±0.91^{ab}	12.13±0.21^{a}	11.06±0.39^{ab}	11.70±0.46^{b}	------
E. coli	11.63±0.23^{c}	15.53±0.14^{a}	16.46±0.34^{a}	17.29±0.44^{ab}	------

Conclusion

The present study was concluded that the tannin, alkaloids, saponins, flavonoids, phenolic, triterpenoids constitute major classes of phytoconstituents of this plant. The plant contains a wide range of phytochemical substances and they credited with various pharmacological properties. Among the four different solvent used, methanol solvent was the most suitable for various phytochemical extractions present in *S. trilobata*. The components identified from the methanolic leaf extract of *S. trlobata* through GC-MS analysis are having more biological activity. Similar reports also observed by Darah *et al.,* 2013. One of the phytochemical components present in the methanolic leaf extract of S. trilobata was Squalene (6.51%), now the researchers plan to use natural oil called squalene to help develop their COVID 19 vaccine. Thus preliminary phytochemical analysis revealed that the presence of various chemical constituents which possess a broad spectrum of biological activity. Hence the plant could be used as an effective drug in the management of various diseases.

The methanolic leaf extracts showed 10.73±0.2, 11.83±0.37, 11.06±0.39, 16.46±0.34 mm zone of inhibition against Staphylococcus *aureus, S. epidermidis, Vibrio cholera and E. coli* respectively in 100 µM concentrations. The leaves extracts of *S. trilobata* also possess the best effect at 100µM concentration with inhibitory zones of 16.46 and 11.63 mm for *E. coli* and *S. epidermidis* respectively. These results were close to inhibition zones of commercial antibiotic of ampicillin at the concentration of 100 µM. The finding in this study has given the strong basic foundation for further purification and structural analysis of the fractions of methanolic leaves extracts.

Acknowledgements

The authors wish to thank the University Grants Commission, New Delhi for providing financial assistance to carry out the Major Research Project on *Cleome gynandra* L. (F. No.: 43-143/2014(SR) dated 21.07.2015, University Grants Commission, New Delhi).

Reference

Balekar N, Nakpheng T Katkam NG, Srichana T. 2012. Wound healing activity of ent-kaura-9(11), 16-dien-19-oic acid isolated from *Wedelia trilobata* (L.) leaves. Phytochemical and Pharmacol. Review **41(3),** 590-605.

Bauer AW, Kirby WMM, Serris JC, Turck M. 1966. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology **45,** 493-496.

Block LC, Santos ARS, Souza MM, Scheidt C, Yunes RA, Santos MA, Monache FD, Filho F. 1998. Chemical and pharmacological examination of antinociceptive constituents of *Wedelia paludosa*, J. Ethnopharmacol **61,** 85-89.

Buddhakala N, Talubmook C. 2020. Toxicity and antidiabetic activity of ethanolic extract of *Sphagneticola trilobata* (L.) Pruski flower in rats. J Ethnopharmacol **262,** 113-128.

Chethan J, Sampath Kumara KK, Niranjana SR, Prakash HS. 2012. Evaluation of antioxidant and antibacterial activities of methanolic flower extract of *Wedelia trilobata* (L.) Hitch. African Journal of Biotechnology **11(41),** 9829-9834.

Coe FG, Anderson GJ. 1996. Screening of medicinal plants used by the Garifuna of eastern Nicaragua for bioactive compounds, J. Ethnopharmacol **53,** 29-50.

Dai ZC, Wang XY, QSS, Cai HH, Sun JF, Huang P, Du DL. 2016. Effects of leaf litter on interspecific competitive ability of the invasive plant *Wedelia trilobata*. Ecological research **31(3),** 367-374.

Darah I, Lim SH, Nithianantham K. 2013. Effects of methanol extract of *Wedelia chinensis* Osbeck (Asteraceae) leaves against pathogenic bacteria with emphasise on *Bacillus cereus*. Indian J Pharm. Sci. **75(5),** 533-539

Govindappa M, Naga SS, Poojashri MN, Sadanana TS, Chandrappa CP, Gustavo S, Sharanappa P, Kumar ANV. 2011b. Antimicrobial, antioxidant and in vitro anti-inflammatory activity and phytochemical screening of water extract of *Wedelia trilobata* (L.) Hitchc. Journal of Medicinal Plants Research **5(24),** 5718-5729.

Govindappa M, Naga SS, Poojashri MN, Sadanana TS, Chandrappa CP. 2011a. Antimicrobial, antioxidant and *in vitro* antiinflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.) Hitchc. J of Pharmacog Phytoth **3(3),** 43-51.

Harborne AJ. 1998. Phytochemical methods a guide to modern techniques of plant analysis. Springer Science.

Harborne JB, Williams CA. 2000. Advances in flavonoids research since 1992. Phytochemistry **55,** 481-504.

Havsteen BH. 2002. The Biochemistry and Medical Significance of the Flavonoids. Pharmacol. Ther. **96(2-3),** 67-202.

Husain N, Kumar A. 2017. Characterization of antioxidant property of root extract of *Sphagneticola trilobata* in recovery of oxidative stress. Indian J. Sci. Res. **12,** 116-20.

Li Donghai Liang, Zhenyi Li Donghai Guo, Mofei Zhou, Jing Yang, Xiaobo, Xu Jing. 2012. Study on the chemical composition and extraction technology optimization of essential oil from *Wedelia trilobata* (L.) Hitchc. African Journal of Biotechnology **11(20),** 4513-4517.

Mardina V, Halimatussakdiah H, Harmawan T, Ilyas S, Tanjung M, Aulya W, Nasution A. 2020a. Preliminary phytochemical screening of different solvent extracts of flower and whole plant of *Wedelia biflora*. IOP Conf Ser Mater Sci Eng **725,** 1-7

Mardina V, Ilyas S, Harmawan T, Halimatussakdiah H, Tanjung M. 2020b. Antioxidant and cytotoxic activities of the ethyl acetate extract of *Sphagneticola trilobata* (L.) J.F. Pruski on MCF-7 breast cancer cell. J Adv Pharm Technol Res **11,** 123-7.

Mardina V, Mastura, Hamdani, Sufriadi E. 2020c. Flower of *Sphagneticola trilobata* (L.) Pruski JF, from Aceh, Indonesia: Antioxidant and Cytotoxic Activity on HeLa Cells. IOP Conf. Ser.: Mater. Sci. Eng. **1007,** 1-7.

Meena AK, Rao MM, Meena RP, Panda P, Renu. 2011. Pharmacological and Phytochemical Evidences for the Plants of *Wedelia* Genus. A Review. Asian J. Pharm. Res **1(1),** 712.

Mishra P, Singh VK, Garg N, Parvez S, Yadav N, Hwisa KI, Molvi SM, AlSharif BZ, Awen RL, Khosa. 2011. Phytochemical screening and anticonvulsant activity of *Wedelia chinensis*. International Journal of Pharmaceutical Sciences and Research **2(1),** 39-43

Mooza Al-Owaisi, Nora Al-Hadiwi, Shah Alam Khan. 2014. GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of *Moringa peregrina* (Forssk.) Fiori leaves. Asian Pac J Trop Biomed **4(12),** 964-970.

Muhammad Tayyab, Durre Shahwar. 2015. GC-MS analysis of *Cannabis sativa*. *Wedelia trilobata* crude extracts. Phytomedicine **6,** 133-134.

Nie CR, Zeng RS, Luo SM, Li HS, Hong MQ, Cheng LQ. 2004. Allelopathic potentials of *Wedelia trilobata* L. on rice. Acta Agronomica Sinica **30,** 942-946.

Oliver-Bever B. 1986. In: Medicinal plants in Tropical West Africa, Cambridge Univerisity. Press, London 245-267.

Prasanth DSNBK, Lakshmana RA. 2018. Evaluation of Pharmacognostic, Phytochemical and Physicochemical Standards of *Wedelia Trilobata* (L.) Root. Pharm Res **2(1),** 149.

Robards K, Prernzler PD, Tucker G, Swatsitang P, Glover W. 1999. Phenolic compounds and their role in oxidative processes in fruits. Food Chem **66,** 401-36.

Santos-Buelga C, Scalbert A. 2000. Pro anthocyanidins and tannin like compounds nature, occurrence, dietary intake, and effects on nutrition and health. J Sci Food Agric **80,** 1094-1117.

Shanmugam S, Malligeswari KS, Venugopalan R, Shanmugam T. 2011. Estimation of Wedelolactone content in *Wedelia* species by HPTLC technique J. Pharm. Res **4,** 193-194.

Shanmuganathan T, Karthikeyan AVP. 2016. Antimicrobial activity of in vivo and in vitro plants of *Cleome gynandra* L. World J Phar Pharm Sci **5(7),** 948-953. ISSN No. 2278 – 4357

Sunita Kanikaram, Hari Babu Bollikolla, Swarna Latha Kurapati, Ramya Krishna Pallapatti. 2018. A Quantitative estimation of phytochemicals, anti-diabetic and antioxidant activities of crude extracts of *sphagneticola trilobata* (L) and *adathoda vasica* Linn. J. Nat. Prod. Res **4(1),** 155-159.

Taddei A, Rosas-Romero AJ. 1999. Antimicrobial activity of *Wedelia trilobata* crude extracts. Phytomedicine **6(2),** 133-134.

Teo BS, Gan RY, Abdul Aziz S, Sirirak T, Mohd Asmani MF, Yusuf E. 2021. *In vitro* evaluation of antioxidant and antibacterial activities of *Eucheuma cottonii* extract and its *in vivo* evaluation of the wound-healing activity in mice. J Cosmet Dermatol **20,** 993-1001

Toppo KI, Gupta S, Karkun D, Agrwal S, Kumar A. 2013. Antimicrobial activity of *Sphagneticola trilobata* (L) pruski, against some human pathogenic bacteria and fungi. The bioscan **8(2),** 695-700.

Trease GE, Evans WC. 1989. In: Pharmacognosy. Braille Tiridel Ed. 2nd Edition, Macmillan publishers.

Verma R, Rajendra S, Padaliaa C, Chauhana A, Sundaresan V. 2014. Essential oil composition of *Sphagneticola trilobata* (L.) Pruski from India. Journal of Essential Oil Research **26(1),** 29-33.

Wendels S, Avérous L. 2021. Biobased polyurethanes for biomedical applications. Bioact Mater **6,** 1083-106.

Widiyowati II, Wardani R, Azmi N, Sukemi S. 2020. DPPH radical scavenging activity of methanol extract of *Wedelia trilobata* flower from Samarinda City, Indonesia. MCTrops **1,** 24-29.

Zhang YH, Liu MF, Ling TJ, wei Xiao Y, South CBG. 2004. Allelopathic sesquiterpene laetoes iron *Wedelia trilobata*. Journal of Tropical and Sub-tropical Botany **12(6),** 533-537.