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Nutrition and Physiology

Phytochemical, pharmacological and tissue culture applications of *Wedelia* spp. – A review

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Abstract. *Wedelia spp. belonging to the family asteraceae whereby most of the species such as W. chinensis (Osbeck) Merr., W. paludosa (Blume), W. trilobata (L.) Hitchc, W. calendulacea (L.) Less., W. prostrata Dalzell & A.Gibson etc. had been traditionally used by the ancient people for medicinal purposes. Due to that, extensive studies regarding the phytochemical and pharmacological aspects of this genus have been carried out for six decades. Most of the studies indicated that the plants from this genus have potential as medicinal herbs to treat diseases such as cancer, diabetes, heart and liver failure, etc. In this paper the phytochemical and pharmacological aspects of some plants of Wedelia genus are reviewed. Tissue culture applications of this genus are not extensively studied, thus in the present review some in vitro results from these investigations are also presented.*

Keywords: *Wedelia* spp., ethnobotany, phytochemistry, pharmacology, tissue culture

Introduction

Plants from *Wedelia* genus have gained a lot of attention since six decades ago due to their ethnobotanical properties. Long ago before the plants had been studied scientifically, the ancient people had used these plants to treat some kinds of illnesses from skin disorders, bacterial infections and even inner body sickness such as heart diseases and liver failure. Leaves of *W. chinensis* (Osbeck) Merr. were reported to be effective to be used as grey hair dyeing and promoting hair growth. The juice from the leaves could serve as a tonic for several illnesses such as coughs, skin diseases, alopecia and cephalgia. The decoction from seeds, flowers and leaves was used in treatment of uterine haemorrhage and menorrhagia (Meena et al., 2011). *W. biflora* (L.) DC. Leaves had also been widely used as a traditional prescription. The leaves juice, when combined with ginger juice could serve as a great prescription for flatulence (Meena et al., 2011). The crushed leaves of *W. biflora* can be used to treat ulcers, cut, sore, and varicose veins in poultice form (Biswas et al., 2013). As for the decoction, it was recommended to be used as an antiperiodic in malaria and haematuria and could also be prescribed for stomach ache (Meena et al., 2011). Crushed leaves of *W. trilobata* Linn. were also used as poultice to treat cuts, ulcers and sore. The decoction from leaves was able to alleviate the symptoms of colds and fever. Apart from that, the decoction was also useful in treatment of hepatitis, microbial infections and able to clear the uterus after birth (Huang et al., 2006). The purpose of the present paper was to make analysis of the recent literature sources referred to different aspects of phytochemical, pharmacological and tissue culture applications of *Wedelia* spp.

Phytochemical studies

Phytoconstituents of plants play crucial role in determining the functions and benefits of the plants. Most of the phytoconstituents are very beneficial in medicinal industry and could serve as new alternatives for existing chemical-based medicines. The phytoconstituents and their chemical structures present in *Wedelia* spp. have been summarized in Table 1.

In a study carried out by Mishra et al. (2011), it has been revealed that the ethanolic extract of whole *W. chinensis* plant contains glycosides, alkaloids and flavonoids while the petroleum ether extract consists of steroids and the aqueous extract contains glycosides and saponins. The chemical components of *W. chinensis* aerial parts were then isolated and identified (Li et al., 2012). The compounds present were (3β)-oleanolic acid 3-(β-D-glucopyranosiduronic acid 6-methyl ester); and (3β)-3-hydroxy-30-noroleana-12.20(29)-dien-28-oic acid 3-(β-D-glucopyranosiduronic acid) 6-methyl ester. Their structures were elucidated using their spectral values (Li et al., 2012). Another study by Banu and Nagarajan (2013) revealed that the bioactive compound of *W. chinensis* (Osbeck) Merrill leaves extract contains 2-tridecanone, n-(methyoxyphenylmethylene) carbamic acid ethyl ester and 9.12.15-octadecatrienoic acid methyl ester.

A similar study on the analysis of phytoconstituents of *W. biflora* volatile oil was conducted by using solid-phase microextraction and GC-MS method (Yang et al., 2013). A total of 68 compounds were identified and most were sesquiterpenoids. The main phytoconstituent were:

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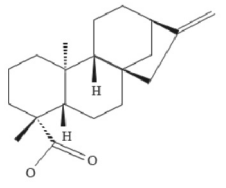
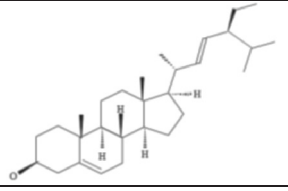
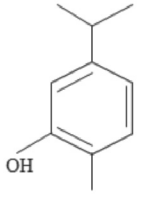
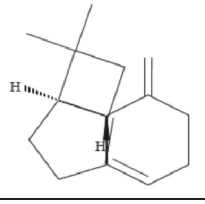
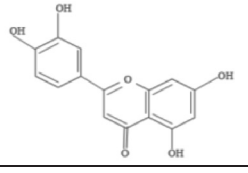
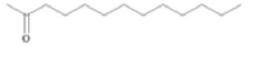
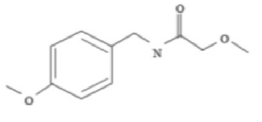
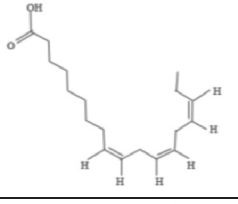
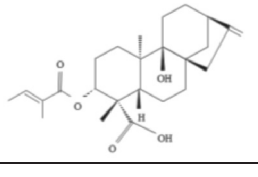
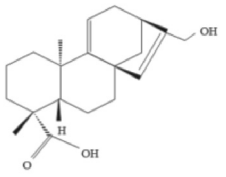
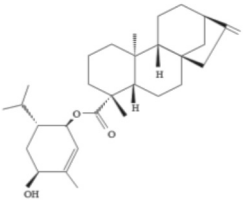
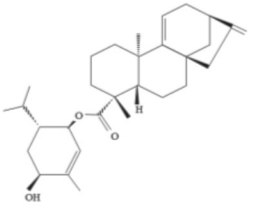
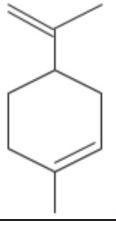
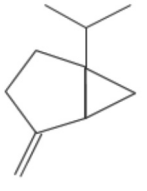
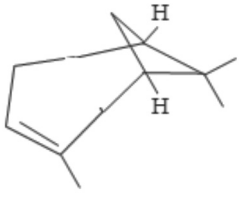
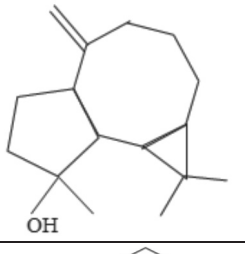
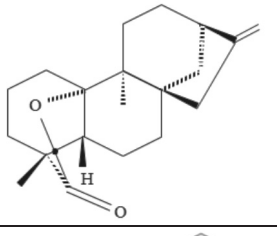
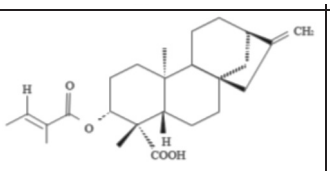
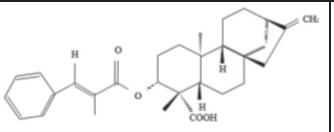
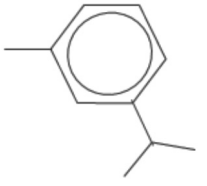
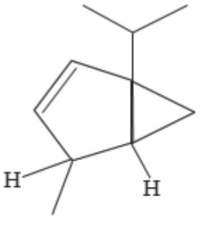
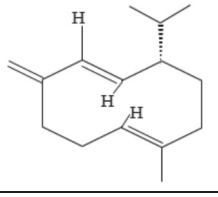
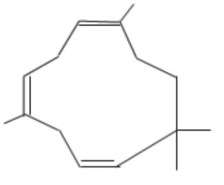
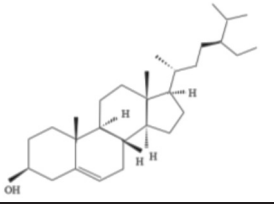
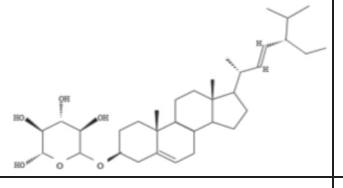
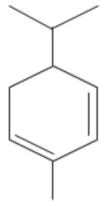
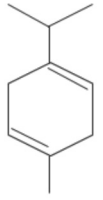
Table 1. Phytochemicals in *Wedelia* spp. with their chemical structures and pharmacological effects

No.	Phytochemicals	Chemical structures	Pharmacological effects	Species	References
1.	Kaurenoic acid		Antinociceptive, anti-inflammatory, antimicrobial, hypoglycemic and antioxidant activities	*W. paludosa*	Block et al., 1998 Sartori et al., 2003 Bresciani et al., 2004
2.	Stigmasterol		Antinociceptive, anti-inflammatory, antimicrobial and antioxidant activities	*W. prostrata, W. biflora, W. paludosa*	Block et al.,1998 Filho et al., 2004
3.	Carvacrol		Antimicrobial and anti-inflammatory activities	*W. biflora*	Manjamalai et al., 2013
4.	t-caryophyllene		Antimicrobial and anti-inflammatory activities	*W. biflora*	Manjamalai et al.2013
5.	Luteolin		Antifungal activity	*W. paludosa*	Sartori et al, 2003
6.	2-Tridecanone		Antimicrobial, anti-tumor, and antioxidant properties	*W. chinensis*	Banu and Nagarajan, 2013
7.	n-(methoxyphenyl-ethylene) carbamicacid ethyl-ester		Antimicrobial, anti-tumor, and antioxidant properties	*W. chinensis*	Banu and Nagarajan, 2013
8.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)		Antimicrobial, anti-tumor, and antioxidant properties	*W. chinensis*	Banu and Nagarajan, 2013
9.	3α-tigloyloxyptero-kaurene L3		Antimicrobial, anti-inflammatory and antioxidant activities	*W. trilobata*	Ma et al., 2013

10.	*ent*-17-hydroxy-kaura-9(11),15-dien-19-oic acid		Antimicrobial, anti-inflammatory and antioxidant activities	*W. trilobata*	Ma et al., 2013
11.	Wedelobatins A		Antimicrobial, anti-inflammatory and antioxidant activities	*W. trilobata*	Ma et al., 2013
12.	Wedelobatins B		Antimicrobial, anti-inflammatory and antioxidant activities	*W. trilobata*	Ma et al., 2013
13.	Limonene		Antimicrobial and antioxidant activities	*W. biflora, W. urticifolia, W. glauca* (Ort.) Hoffman ex Hickens, *W. chinensis*	Bailac et al., 2005 Garg et al., 2005
14.	Sabinene		Antimicrobial and antioxidant activities	*W. glauca* (Ort.) Hoffman ex Hicken	Bailac et al., 2005
15.	α-pinene		Antimicrobial and antioxidant activities	*W. glauca* (Ort.) Hoffman ex Hicken, *W. biflora, W. urticifolia, W. chinensis*	Bailac et al., 2005 Garg et al., 2005
16.	Spathulenol		Antimicrobial and antioxidant activities	*W. chinensis*	Garg et al., 2005
17.	Tetrachyrin		Anti-inflammatory and antioxidant activities	*W. paludosa*	Vieira et al, 2001

18.	3α-tigloyloxykaur-16-en-19-oic acid		Anti-inflammatory and antioxidant activities	*W. paludosa*	Vieira et al., 2001
19.	3α-cinnamoyloxykaur-16-en-19-oic acid		Anti-inflammatory and antioxidant activities	*W. paludosa*	Vieira et al., 2001
20.	1-methyl-3-(1-methylethyl)-benzene		Antimicrobial and anti-inflammatory	*W. biflora*	Yang et al., 2013
21.	4-methyl-1-(1-methylethyl-didehydro deriv.) bicyclo[3.1.0]hexane		Antimicrobial and anti-inflammatory	*W. biflora*	Yang et al., 2013
22.	Germacrene D		Antimicrobial and anti-inflammatory	*W. biflora, W. urticifolia*	Zhu et al., 2012
23.	1,1,4,8-tetramethyl-cis, cis, cis-4,7,10-cycloundecatriene		Antimicrobial and anti-inflammatory	*W. biflora*	Yang et al., 2013
24.	β-sitosterol		Antinociceptive, anti-inflammatory, antimicrobial and antioxidant activities	*W. prostrata*	Zhang et al., 2011
25.	Stigmasterol 3-O-beta-D-glucoside		Antinociceptive, anti-inflammatory, antimicrobial and antioxidant activities	*W. prostrata*	Zhang et al., 2011
26.	n-hexacosanol		Antinociceptive, anti-inflammatory, antimicrobial and antioxidant activities	*W. prostrata*	Zhang et al., 2011

27.	α-phellandrene		Antimicrobial and anti-inflammatory	*W. urticifolia*	Zhu et al., 2012
28.	γ-terpinene		Antimicrobial and anti-inflammatory	*W. urticifolia*	Zhu et al., 2012

• D-limonene;
• 4,11,11-trimethyl-8-methylene-[1R-(1R*,4Z,9S*)]bicyclo[7.2.0]undec-4-ene (β-ca-ryophyllene);
• 1R-alpha-pinene;
• 4-methyl-1-(1-methylethyl-didehydro deriv.) bicyclo[3.1.0]hexane;
• 1-methyl-3-(1-methylethyl)-benzene;
• 1,1,4,8-tetramethyl-cis, cis, cis-4,7,10-cyclo-undecatriene, and
• germacrene D.

In a recent study on the diterpenoids of *W. prostrata*, the chemical constituents were isolated and elucidated by using chromatography and spectroscopic techniques. Some of the compounds isolated and identified were ent-kaur-16-en-19-oic acid; β-sitosterol; stigmasterol; stigmasterol-3-O-beta-D-glucopyranoside and n-hexacosanol (Zhang et al., 2011). In a previous study carried out by Zhu et al. (2015) the chemical composition of essential oils from different parts of *W. urticifolia* were quantified. The most abundant compounds were α-Pinene and D-limonene in flower essential oils, while the leaf essential oils contain α-pinene, d-limonene, α-phellandrene, Y-terpinene and germacrene D. The major compounds in stems essential oils were D-limonene, Y-terpinene, germacrene D, and α-pinene.

Pharmacological studies

Hepatoprotective activity

Emmanuel et al. (2001) studied the methanolic extract of *W. calendulacea* Less. leaves on the paracetamol induced liver damage in Wistar albino rats. A group of compounds, coumestans, present in leaves of *W. calendulacea* exhibited significant hepatoprotective property as it reduced cell membrane disturbances induced *in vivo* by paracetamol. Paracetamol damage to liver raises the serum levels of lactate dehydrogenase (LDH), alanine transaminase (ALT), aspartate transaminase (AST) and acid phosphatase by releasing them into the blood stream. Pre-treatment with *W. calendulacea* leaves extract (with coumestans as main compound) reduced the increased enzyme activities produced by paracetamol. Subsequent recovery towards normalization of these enzymes strongly suggests the possibility of coumestans to accelerate regeneration of parenchyma cells by fixing the hepatocytes.

W. chinensis whole plant ethanolic extract was found to have a significant hepatoprotective activity against carbon tetrachloride (CCl_4). Significant reduction was observed in AST, ALT, alkaline aminotransferase, total protein and total bilirubin tests as compared to standard drug treatment along with normal hepatic cords in a histopathological study (Jalal et al., 2012).

Wound healing and anti-inflammatory activities

The wound healing activity of whole plant methanolic extract from *W. chinensis* was investigated by excising wound of *Albino Wistar* rat. The methanolic extract was found to decrease the period of epithelialization and increased the rate of wound contraction for the excision model. This suggested that the methanolic extract of *W. chinensis* plant contained a potential wound healing agent in medicine (Nomani et al., 2013). Biswas et al. (2013) had also tested ethanolic extracts of *W. biflora* for wound healing property using the same model as Nomani and Kotnala (2013). It was found that ethanolic extract of *W. biflora* resulted in higher wound healing percentage as compared to the control.

Wedelactone, which is a major constituent of *W. chinensis* was tested for its pro-inflammation effects by using a cellular model of lipopolysaccharide (LPS)-induced RAW 264.7 cells. The pro-inflammation activity of wedelactone was measured by several tests and the result showed significant inhibition of protein expression levels of inducible nitric oxide synthase (iNOS) and cyclooxgenase-2 (COX-2) in lipopolysaccharides-induced cells and also inhibited the transcription factors, lipopolysaccharide-induced Nuclear Factor KappaB ($NF_kβ$) p65 activation. It showed that this active compound can be used as anti-inflammatory agent (Yuan et al., 2013). A previous study by Manjamalai et al. (2011) had also proven anti-inflammatory effect of *W. chinensis* leaf extracts.

Leaves extract of *W. trilobata* was also found to have a wound healing property. In the ethyl acetate fraction of *W. trilobata* leaves (ethanol extract) it showed antibacterial and fibroblast stimulatory activities (Balekar et al., 2012). Then, Balekar and his groups further investigated the active component of leaves extracts and isolated grandiflorenic acid (ent-kaura-9(11), 16-dien-19-oic acid) which displayed an-

tibacterial, fibroblast growth stimulation and protective effects against hydrogen peroxide induced injury. Balekar et al. (2013) also investigated the *in vitro* stimulatory effect of grandiflorenic acid found from the leaves of *W. trilobata* (L.) on L929 mouse fibroblast cells. The results showed that the grandiflorenic acid is responsible for the wound healing activity. A further experiment was conducted on BJ human and HaCaT keratinocytes to evaluate the potential of grandiflorenic acid from leaves of *W. trilobata*. They showed a higher viability percentage of BJ human fibroblast and HaCaT keratinocytes. It also induced 100% migration rate in *in vitro* scratch assay and 171.2 µg/mL collagen content as compared to control with BJ human fibroblast. Fibroblast growth stimulation and prolonged inflammatory inhibition phase indicated an evidence of wound healing activity (Balekar et al., 2013).

Analgesic activity

Ethanolic extract of *W. chinensis* has been evaluated for its analgesic effects via hot plate and acetic acid induced writhing methods (Sureshkumar et al., 2006). The evaluation was carried out on Swiss albino rats of both sexes. It was found that the extracts could inhibit the writhing response induced by acetic acid in a dose dependent manner. They suggested the antioxidant activity of phytochemicals such as flavonoids, triterpenoids and steroids present in *W. chinensis* were responsible in significant analgesic activity against early phase (acute paw edema) and late phase (cotton pellet edema) of inflammation model.

Mizokami et al. (2012) studied the analgesic effect of kaurenoic acid from *W. trilobata* on the cytokine production and activation of the NO-cyclic GMP protein kinase G-ATP-sensitive potassium channel signalling pathway in mice by intraperitoneal and oral treatment. The result showed that kaurenoic acid exhibited analgesic effect in the treatment and the plant has a potential to be developed as a therapeutic medicine.

Chemotherapeutic effects

The chemotherapeutic activity of methanol extract from *W. calendulaceae* was studied against 20-methylcholanthrene-induced carcinogenesis in Swiss albino mice. The extract significantly increased the life span of treated mice, reduced the tumor incidence and restored the hematological profiles as compared to the control group (Halder et al., 2011). The chemopreventive effect of *W. calendulacea* ethanolic extract was further studied against DEN induced and Phenobarbital promoted hepatocellular carcinoma in albino rats. It showed that the extract was able to protect DEN and Phenobarbital induced hepatocellular carcinoma in the treated rats. It revealed that the extract can be developed as a potent chemopreventive medicine (Dutta, 2013).

Manjamalai and Grace (2013) explored the chemotherapeutic effect of *Wedelia chinensis* (Osbeck) essential oil against lung metastasis in C57BL/6 mice. It revealed that active compound of *W. chinensis* has a potent chemotherapeutic activity against cancer by significantly increasing the apoptosis cells and suppressing angiogenesis in the treated mice.

Anti-colitis

The activity of anti-colitis of *W. chinensis* different extracts were studied against dextran sulfate sodium-induced acute colitis in C57BL/6 mice. The daily administration of hot water extract was found to produce a significant attenuation of colitis symptoms, reduced shortening of colon length and histopathological damages. It was also observed that *W. chinensis* extract can suppress the Th1 and Th17 (Helper T cell) responses in colon tissues and dendritic cells of treated mice (Huang et al., 2006).

Cardiotonic and anticonvulsant activity

The phytochemical screening of aqueous leaf extract from *W. chinensis* was found to possess cardiac glycosides. It is responsible for the contraction force of the cardiac muscle. It was revealed that the extract of *W. chinensis* has a potential cardiotonic activity without producing cardiac arrest on animal models which are comparable to the marketed drug, Digoxin (Karumuri and Kasagana, 2012).

Anticonvulsant (anti-epileptics) activity of various extracts of whole plant *W. chinensis* in mice was studied by using maximum electroshock-induced and PTZ seizure methods. Both models showed that the ethanolic extract at 750mg/kg dose level exhibited comparable activity to the standard compound, phenytoin (Mishra et al., 2011).

Antioxidant activity

The antioxidant activity from the essential oils of *W. chinensis* were investigated against *in vitro* and *in vivo* Lung Cancer Bearing c57BL/6 in mice (Karumuri and Kasagana, 2012) and it showed a significant correlation between concentrations of *W. chinensis* essential oils and percentage inhibition of free radicals. In a previous study by Senthilkumar et al. (2008), treatment with ethanolic extract of *W. chinensis* was enhancing the antioxidant activity in alloxan induced rats. Therefore, *W. chinensis* can be extensively studied to treat diseases related to free radicals scavenging activities.

The experiment to evaluate the antioxidant activity of methanolic extract from different plant parts of *W. trilobata* was studied *in vitro*. The result showed that the flower part exhibited the highest antioxidant activity when compared to the other plant parts (Jayakumar et al., 2011). The methanolic flower extract of *W. trilobata* (L.) contains antioxidant activity with IC_{50} value of 90µg/ml in DPPH and IC_{50} value of 80 µg/ml radical scavenging assay. They displayed different values due to different mechanisms of radical antioxidant reaction in both assays (Chetan et al., 2012). Apart from that, water extract of leaf and flower of *W. trilobata* had also shown some free radicals scavenging activity (Govindappa et al., 2011).

Essential oil from *W. prostrata* was another source for potential antioxidant agent. Dai et al. (2013) investigated the

antioxidant properties of this essential oil by determining the free radicals-scavenging activity. It was shown that essential oil of *W. prostrata* had moderate antioxidant activity and could be developed further for production of useful medicines.

Antidiabetic activity

Bresciani et al. (2004) had studied the hypoglycemic effect of *W. paludosa* extracts. They found that kaurenic acid was possibly the compound that was responsible for hypoglycemic effect found in that plant. The plant extract was able to lower the blood glucose level in alloxan-induced Wistar rats. Methanolic extract of *W. chinensis* has also been studied for its antidiabetic properties. Nomani and Kotnala (2013) reported that this extract had antihyperglycemic effect in alloxan-induced diabetic rats. The blood glucose levels of diabetic control rats were found to be decreased as compared to normal control rats.

Anti-cancer activity

Methanolic extracts of *W. calendulacea* had been reported to be a potential anti-cancer agent. Gupta et al. (2007) studied the anticancer activity of *W. calendulacea* methanolic extracts against Ehrlich Ascites Carcinoma (EAC) in Swiss albino mice. They found that these extracts increased the life span of EAC treated mice as compared with the EAC bearing mice. Manjamalai and Grace (2013) studied the chemotherapeutic effect of *W. chinensis* (Osbeck) essential oil against lung metastasis in C57BL/6 mice. It was established that active compound of *W. chinensis* has a potent chemotherapeutic activity against cancer by significantly increasing the apoptosis cells and suppressing angiogenesis in the treated mice.

Anti-cancer activities of different extracts from *W. chinensis* have been studied against five human cell cancer lines in inhibiting the nasopharyngeal carcinoma CNE-1 cell growth. The result showed that only one subfraction of ethyl acetate extract (EA6) exhibited cytotoxic activity to nasopharyngeal carcinoma cells (Liu et al., 2013). A pre-clinical study by Tsai et al. (2009) had shown that herbal extract of *W. chinensis* attenuated the growth of prostate cancer cells in nude mice. They found that the potential antitumor compounds were wedelolactone, luteolin and apigenin.

Antimicrobial studies

W. biflora is said to be useful in the treatment of microbial and wound infections. Several studies have been conducted to prove this traditional claim. For instance, a study was conducted on the antifungal activity of essential oil of *W. biflora* against *Candida albicans* in Indonesia. The essential oils from the leaves of *W. biflora* with other plant species found in Aceh Province were tested against *Candida albicans* with concentrations of 1%, 5% and 10%. The results for *W. biflora* showed no zone of inhibitions for antifungal activity (Ginting, 2012). Thus, a recent study on the evaluation of the antimicrobial and wound healing property of ethanolic extract of *W. biflora* was conducted. The results of ethanolic extracts on the tested microorganisms showed maximum inhibition with higher range of the zone of inhibition (ZI) 11.3-21.6mm. The best result for antimicrobial study was achieved against *Candida albicans* with MIC values 39µg/ml as compared to the control, 4.8-19.50µ/ml .

The results of antibacterial activity revealed that the methanolic extract of *W. chinensis* leaves exhibited a potent antibacterial activity against Gram positive bacteria (*Bacillus cereus, Bacillus subtilis* and *Staphylococcus aureus*) as compared to the Gram negative bacteria (*Escherichia coli, Proteus rettgeri* and *Pseudomonas aeruginosa*). This study showed that higher concentration of extracts exhibited significant inhibition of bacterial cells growth (Darah et al., 2013). The antibacterial and antifungal activities of ethanolic and hexane extracts of *W. chinensis* leaves were investigated against pathogenic bacteria and fungi. Both extracts exhibited significant result in antibacterial and antifungal study. For antifungal activity evaluation, the extract was found to be effective against four strains of fungi (*Aspergillus niger, Aspergillus flavus, Candida albicans, Alternaria alternate*) (Das et al., 2013).

The phytochemistry and antimicrobial effect of the essential oil from *W. prostrata* were studied against 10 different strains of microorganisms. The results showed that the essential oil possess a potent antimicrobial activity against the tested microorganisms. The antimicrobial activity of the main bioactive compounds, limonene and alpha-pinene were also evaluated and α-pinene exhibited higher antimicrobial activity as compared to the limonene compound (Dai et al., 2013). The antimicrobial activity of *W. trilobata* from different plant parts were investigated against 7 bacterial strains using agar diffusion method. The flower part showed maximum inhibition against *Salmonella pratyphi* and *Bacillus cereus* (Jayakumar et al., 2011).

Plant tissue culture

Plant tissue culture technique is the best method to study the cellular behavior of plants and also for mass propagation of important species. In this technique, only small portions of plants are needed to propagate into whole plants or callus. Micropropagation to produce a new whole plant is useful in bulk production of plants in a shorter period of time. Meanwhile, callus culture is very useful in secondary metabolite production and analysis.

In *W. chinensis*, micropropagation studies were carried out by using nodal segments as explants; which indicated that Murashige and Skoog (MS) medium supplemented with 6-benzylaminopurine (BAP) and indole-3-butyric acid (IBA) was the best for axillary bud multiplication and rooting, respectively (Martin et al., 2003). Meanwhile, Rahman and Bhadra (Rahman and Bhadra, 2011) had established a rapid and reproducible propagation method for *W. chinensis* by using different types of explants. They obtained the highest number of multiple shoots from nodal segments in MS basal medium fortified with BAP (3.0mg/L) and indole-3-acetic acid (IAA, 0.5mg/L). Rooting of regenerated shoots was best ob-

served on half strength MS medium supplemented with IBA (2.0mg/L).

In a study by Agarwala et al. (2010), they used shoot tips and nodes as explants for clonal propagation on MS medium supplemented with different concentrations of auxins, cytokinins and gibberellic acid. Best response for shoot elongation was observed on shoot tips cultured in MS medium containing a combination of BAP (1.0mg/L) and α-napththaleneacetic acid (NAA, 1.5mg/L) and best response for shooting and rhizogenesis was observed in MS medium fortified with 1.0mg/L BAP and 1.5mg/L NAA.

In vitro callus induction from *W. trilobata* was obtained by using leaf, shoot tips and nodal segments as explants. The best result for callusing was achieved in leaf explants supplemented with 2.0mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and shoot tips explants with 1mg/L 2,4-D (Thakur et al., 2011). A new potential method for *in vitro* propagation of *W. trilobata* was developed using extracellular products (EP) and biomass water extracts (BWE) of *Phormidium subincrustatum*. This cyanobacterial extracts serve as an organic source to the MS media. The best medium for callus growth was observed on MS medium supplemented with BWE as compared to the control. It showed a significant increase in shoot length and callus volume within 15 days of culture. It was found that MS medium fortified with cyanobacterial extracellular product is cost effective and can replace the widely used chemically synthesized plant growth regulators in MS media (Keerthiga et al., 2012).

In the present study, *in vitro* callus induction protocol was optimized by using stem and leaf explants of *W. biflora*. It was observed that stem explants had better callusing response than leaf explants in various concentrations of auxins (NAA, IBA, 2,4-D and IAA) and cytokinins (BAP and Kinetin) (Figure 1A). The regeneration protocols of *W. biflora* was also established and resulted in multiple shoots propagation (more than 30 shoots per explant) by using MS medium supplemented with BAP (1.0mg/L) alone (Figure 1B). The propagated shoots were successfully rooted in half-strength MS media fortified with IBA and the acclimatization success was almost 100%. Our results for this current study are very useful in exploration of *W. biflora* phytoconstituent for medicinal purposes.

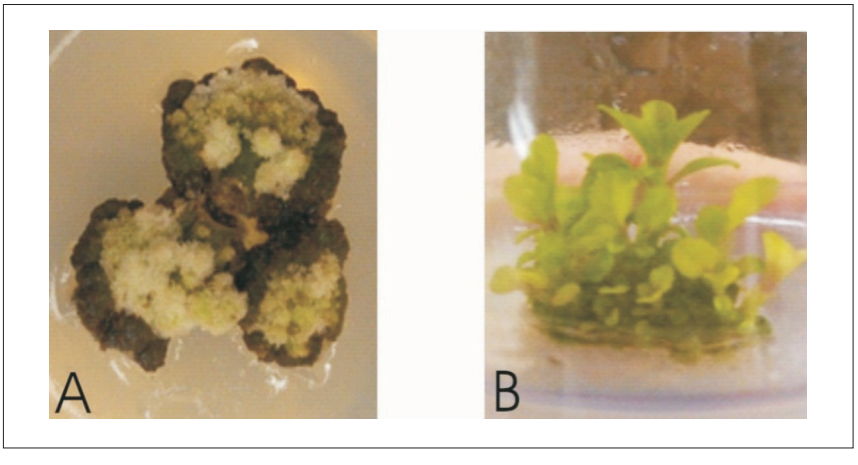


Figure 1. A: Callus obtained from stem explant formation of *W. biflora* grown on MS medium supplemented with a combination of 2.0mg/L NAA and 3.5mg/L BAP. B: Multiple shoots formation from *W. biflora* cultured on MS medium supplemented with 1 mg/L BAP.

Conclusion

Wedelia spp. had been proven to be very effective as traditional medicine without any major side effects. We highlighted the traditional uses, phytochemistry, pharmacology of *Wedelia* spp. and potential mass propagation through tissue culture. Extracts and phytoconstituents from plants from this genus have shown to produce different pharmacological response, which includes hepatoprotective effects, anti-inflammatory, analgesic, chemotherapeutic, anti-colitis, cardiotonic effect, anticonvulsant and antidiabetic. Meanwhile, they were also reported to be very potent antioxidant and anticancer agents as well as that some tissue culture achievements had also been studied of the phytoconstituents of plants via *in vitro* methods. Hence, this review serves to give all the scientific information regarding some useful plants in *Wedelia* genus.

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