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Andrographis paniculata Nees (*Kalmegh*): A Review on Its Antibacterial Activities and Phytocompounds

Chandni Tandon[1*], Priti Mathur[1] and Manodeep Sen[2]

[1]Amity Institute of Biotechnology, Amity University, Uttar Pradesh, Lucknow- 226028, India.
[2]Department of Microbiology, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow-226010, India.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Aims: To study the number of researches performed in the area of antibacterial activities and phytocompounds of *Andrographis paniculata.*
Study Design: The present scenario of increasing rate of multi-drug resistance to pathogenic organisms has necessitated a search for antimicrobial substances from other sources especially from plants. *Andrographis paniculata* is a potent medicinal plant in the Indian system of medicine belonging to the family Acanthaceae. It is known to exhibit significant antibacterial properties and commonly called as king of bitter (English), kalmegh (Hindi) and Chiretta (Urdu). The plant contains variety of chemical constituents but andrographolides is the major constituent of this plant which is believed to be responsible for the most biological activities.
Results: Studies showed that leaves are the most promising part as a source for antibacterial agents. Ethanol, chloroform and methanol were came out to be the most promising solvents to extract phytocompounds. 3-O-β-D-glucosyl-14-deoxyandrographolide and 14-deoxyandrographolide are the two compounds isolated from *Andrographis paniculata* showing broad spectrum antibacterial activities.

**Corresponding author: E-mail: chandni.tndn@gmail.com;*

Conclusion: From this review it is clear that the studied plant can be used for developing drugs in order to fight with various infectious diseases.

Keywords: Andrographis paniculata; multi-drug resistance; andrographolide; antibacterial activities; phytocompounds.

1. INTRODUCTION

Infectious diseases are accounting approximately half of the deaths throughout the world. About 50-75% of hospital deaths are reported due to infectious diseases [1]. These numbers are increasing rapidly due to the development of bacterial resistance to certain antibiotics, so there is an urgent need of research for investigating plants as an alternative source to existing drugs in order to fight with the health problems. Some plants have shown the ability to fight against bacterial resistance problems and this has led the scientists to isolate active compounds from it and find out their mechanism of action [2].

Andrographis paniculata is an annual herbaceous plant belonging to the family Acanthaceae. It is popular worldwide with the name of king of bitter (English) because of its extremely bitter taste, kalmegh (Hindi) and Chiretta (Urdu). It is native to India and Srilanka but widely cultivated in southern Asia, Scandinavia, China and some parts of Europe. The plant has a strong traditional usage from safety point of view because of this it has been used since long time without any known toxicity. It grows erect in moist shady places, running ½ to 1 meter in height with glabrous leaves up to 8 cm long, 2.5 cm broad and white flowers with petals having purple spots on it. The stem is dark green, 2-6 mm in diameter. Although all parts of the plant have been reported traditionally at times, the leaves are the most common medicinal part of this plant. The plant is commonly used to get rid of body heat, dispel toxins from the body, treat common cold, acidity, liver complaints, upper respiratory tract infections such as sinusitis and fever [3,4], it also act as antidote against snake's and insect's poisons [5]. Despite of antibacterial property, the plant is also known for its anti-inflammatory [6,7], antipyretic [8], anti-viral [9], anti-hyperglycemic [10], antioxidant [11] properties etc. The primary medicinal component of *Andrographis paniculata* is andrographolide, which is a 'diterpene lactone' water soluble substance and has been known to exhibit anticancer [12], anti HIV [13], cardioprotective [14] and hepatoprotective [15] properties. In this review we have focused on the antibacterial properties and phytocompounds of *Andrographis paniculata* studied by different scientists.

1.1 Antibacterial Activity of *Andrographis paniculata*

Sukesh et al. [16], investigated the antibacterial activity of *Andrographis paniculata* by filter paper disc-agar diffusion procedure against common bacterial pathogens: *Pseudomonas aeruginosa, Clostridium perfringens, Serratia marcescens, Bacillus subtilis, Enterobacter aerogenes, Shigella flexneri, Staphylococcus aureus* and *Salmonella typhi.* The plant was extracted using hexane and chloroform. Both the extracts were found inhibitory to all the bacterial pathogens, but the highest inhibition was found towards Methicillin resistant *S. aureus.* Phytochemical study revealed the presence of steroids/ terpenoids and coumarins in the extracts. Further TLC analysis showed five compounds in hexane extract whereas 22 compounds in Chloroform extract. The aqueous and methanol extracts of the leaves, stem, root and whole plant of *Andrographis paniculata* were studied by Kumar et al. [17], for their antibacterial activity by agar-well diffusion method against *Staphylococcus aureus, Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris*. It was observed that, methanol extracts of whole plant and leaves showed the significant antibacterial activity against the tested organisms, but the aqueous extracts of the same showed less activity. No bacterial activity was recorded with aqueous extracts of stem and root. Youhong et al. [18], investigated the antimicrobial activity of aqueous and two ethanolic extracts (80% and 100%) of *Andrographis paniculata* whole plant against nine bacterial species including *Salmonella typhimurium, Escherichia coli, Shigella sonnei, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumonia, Streptococcus pyogenes, Legionella pneumophila* and *Bordetella pertussis* using the disc diffusion method. Of all tested pathogens the antimicrobial activity of the two ethanolic

Andrographis paniculata extracts was observed for only *Legionella pneumophila* and *Bordetella pertussis*

In vitro antibacterial activity of hexane and methanolic extracts of the roots of *Andrographis paniculata* were investigated by Radhika et al. [19], against *Bacillus pumilus, Bacillus subtilis, Escherichia coli* and *Proteus vulgaris* using agar well diffusion technique. Both the extracts were found to inhibit the growth of all tested bacteria. Sule et al. [20], evaluated the antibacterial activity of dichloromethane, methanol and aqueous extracts of whole plant of *Andrographis paniculata* against skin disease causing pathogens such as *Staphylococcus saprophyticus, Staphylococcus epidermis, Staphylococcus aureus, Streptococcus pyogenes, Bacillus anthracis, Micrococcus luteus, Enterococcus faecalis, Proteus mirabilis, Proteus vulgaris, Klebsiella pneumoniae, Neisseria meningitis* and *Pseudomonas aeruginosa* using the disc diffusion method. The extracts showed significant antibacterial activities against the tested pathogens. Phytochemical analysis revealed the presence of terpenoids, tannins, flavonoids, saponins, alkaloids, amino acids and steroids in the extracts. Doss and Kalaichelvan, [21], evaluated the antibacterial activity of *Andrographis paniculata* leaf ethanolic extract against *Micrococcus luteus, Staphylococcus aureus, Escherichia coli and Klebsiella pneumonia* using agar well diffusion method. Results showed that the extract was able to inhibit *Escherichia coli, Staphylococcus aureus and Micrococcus luteus* but not *Klebsiella pneumonia.*

The antibacterial activity of leaf extracts of *Andrographis paniculata* were evaluated by Hannah et al. [22], against forty-nine bacterial isolates (22 *Staphylococcus aureus*, 8 *Escherichia coli*, 6 *Klebsiella pneumonia* and 2 *Klebsiella oxytoca*, 2 *Proteus vulgaris*, 2 *Proteus mirabilis*, 2 *Salmonella typhi*, 3 *Pseudomonas aeruginosa*, 1 each of *Acinetobacter baumannii* and *Yersinia intermidis*) using agar well diffusion method. Leaves were extracted using raw juice, acetone, methanol and ethanol. Results showed that the extracts did not exhibit appreciable antibacterial activity. The antibacterial activity of petroleum ether, acetone, chloroform and methanol leaves and stems extracts of *Andrographis paniculata* were studied by Radha et al. [23], against strains of *Enterococcus faecalis, Streptococcus pyogenes, Klebsiella pneumonia* and *Proteus vulgaris* using disc diffusion method. All the extracts showed inhibitory effect against the tested organisms, but the most significant antibacterial activity was found against *Enterococcus faecalis.* Phytochemical analysis revealed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, tannins and saponins in the extracts. In-vitro antibacterial activity of dichloromethane, methanol and aqueous extracts of whole plant of *Andrographis paniculata* were evaluated by Sule et al. [24], against *Staphylococcus saprophyticus, Staphylococcus epidermis, Staphylococcus aureus, Streptococcus pyogenes, Bacillus anthracis, Micrococcus luteus, Proteus mirabilis, Proteus vulgaris, Neisseria meningitis* and *Pseudomonas aeruginosa* using disc diffusion method. The extracts showed significant antibacterial activities against both gram-positive and gram-negative bacterial strains tested. Time-kill experiments indicated that the extracts have bactericidal characteristic against most of the gram positive bacteria and bacteriostatic activity against both gram negative and gram positive bacteria. Phytochemical screening revealed the presence of terpenoidal and flavonoidal compounds in all the extracts.

Kataky and Handique, [25], evaluated the antibacterial activity of eight-months old micropropagated plantlets of *Andrographis paniculata* against *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* using agar well diffusion method. The dried parts (leaf, stem and root) of the plant were extracted with chloroform, acetone, ethyl acetate, DMSO and petroleum ether. Results showed that the chloroform extract exhibited strong inhibitory activity with all the microbes tested. Out of the five microbial test organisms *Staphylococcus aureus* was the most susceptible. Phytochemical analysis revealed the presence of carbohydrates, proteins, flavonoids, phenolics, saponin and alkaloids in the dried powder. The antibacterial activity of ethanol extract of the aerial part of *Andrographis paniculata* were studied by Mishra et al. [26], against *Escherichia coli* K 12 ROW, *Staphylococcus aureus* 29737, *Staphylococcus aureus* ML 59, *Shigella boydii* 8, *Salmonella typhimurium* NCTC 74, *Shigella sonnei* 2, *Vibrio cholerae* 854, *Vibrio cholerae* 811, *Salmonella typhi* 59, *Vibrio alginolyteus* and *Bacillus licheniformis* 10341 using disc diffusion method. Results revealed that the ethanol extract showed potent antibacterial activity against the tested

pathogens. Anitha et al. [27], evaluated the antibacterial effect of leaf, roots and shoots extracts of normal and tissue cultured plants of *Andrographis paniculata* using different solvents like petroleum ether, ethanol, methanol and aqueous against *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus vulgaris*. Results of agar well diffusion method indicated that ethanolic and methanolic leaf extracts of both samples exhibited significant antibacterial activity against the tested bacteria. Also methanolic leaf extracts of tissue cultured plants showed better antibacterial activity than the normal plant.

Hosamani et al. [28], identified antibacterial activity of leaf extract of *Andrographis paniculata* using different solvent like chloroform, acetone, ethanol and water against *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The results of the disc diffusion method showed that the acetone and ethanol extracts was most active against *Staphylococcus aureus* and *Bacillus subtilis.* Phytochemical analysis revealed the presence of saponins, flavonoids and phenolic compounds in the extracts. The antibacterial activity of flower extract of *Andrographis paniculata* were studied by Suneetha and Ravi, using different solvents like chloroform, acetone [29], ethanol and water against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus* sp., *Micrococcus luteus* and *Bacillus* sp. by disc diffusion method. Out of the four extract used, acetone and ethanol extracts were found to be most active against *Staphylococcus aureus* and *Bacillus subtilis.* In-vitro antibacterial efficacy of ethanolic leaf extract of *Andrographis paniculata* was assessed by Mishra et al. [30], using agar cup plate method against *Proteus vulgaris, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.* Results showed that the plant extract exhibited significant antibacterial activity against the tested pathogens. The antimicrobial activity of aerial parts of *Andrographis paniculata* was described by Roy et al. [31], against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhimurium, Enterobacter cloacae*, *Staphylococcus aureus, Bacillus subtilis, Enterobacter faecalis* and *Staphylococcus epidermidis*. The extract of the plant was prepared in chloroform and chloroform+HCL, respectively. The results of agar well diffusion method showed that the chloroform extract exhibited better antibacterial activity as compare to chloroform+HCL extract. To find out the nature of the compounds responsible for the antimicrobial activity, GC-MS analysis was performed which revealed the presence of phenols, aromatic carboxylic acids and esters in the extracts.

In vitro antibacterial activity of the chloroform extracts of the root and the stem of *Andrographis paniculata* was screened by Parvataneni and Koduru, [32], against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris* by cup plate method. All the extracts showed considerable antibacterial activity against the tested pathogens. Bobbarala et al. [33], determine the potential antimicrobial activity of hexane, chloroform and methanolic extracts of whole plant of *Andrographis paniculata* against *Erwinia caratovora*, *Pseudomonas marginales, Pseudomonas syringae, Pseudomonas aeruginosa and Xanthomonas compestris* using agar well diffusion method. All the extracts showed significant antibacterial activity against the tested pathogens. The antibacterial activity of chloroform extract of *Andrographis paniculata* leaves and roots was identified by Sivananthan and Elamaran, [34], against *Staphylococcus aureus*. Results of agar well diffusion assay showed that the plant do possess some antibacterial property against the strain tested. Arunadevi et al. [35], investigated the antibacterial activity of methanolic and water extract of *Andrographis paniculata* leaves, stem, branches, seed, root and buds both in fresh and dried form against *Bacillus subtilis, Salmonella typhi, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Enterobacter faecalis* and *Pseudomonas aeruginosa* using agar well diffusion method. The results showed that the crude extracts of the leaves, stem and branches of the plant exhibited maximum antibacterial activity. A Thin layer chromatography was also performed to identify the small molecular compounds in the extracts, the results of which showed the presence of 29 compounds. The antibacterial potential of water, methanol, ethanol and chloroform extracts of *Andrographis paniculata* leaf were studied by Malahubban et al. [36], against *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Salmonella enteric*. The results of the disc diffusion method indicated that the methanolic extract of the plant exhibited the strongest inhibitory effects across the bacteria tested. Phytochemical screening of the extracts revealed the presence of Alkaloids, Saponins, Flavanoids, Tannins, Terpenoids and Steroids. HPLC analysis was also performed to

determine the andrographolide in the extracts, which showed that the methanolic extract of *A. paniculata* leaves gave the highest amounts of andrographolide. Mamun et al. [37], investigated a cost effective protocol for rapid *in vitro* regeneration of *Andrographis paniculata* (Kalmegh) and also the antibacterial activity of its crude protein extracts against *Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus, Bacillus subtilis* and *Mycobacterium smegmatis* using disc diffusion method. The *in vitro* shoot formation, shoot multiplication, root induction and establishment of whole plantlets from shoot tips and nodal segment of Kalmegh was undertaken using MS media supplemented with BAP, Kn NAA and IBA, either alone or in combination. BAP alone showed maximum (100%) shoot regeneration from nodal segment at a concentration of 0.5 mg/l. In combination, medium having 0.5 mg/l BAP + 0.1 mg/l NAA was found to be best for auxillary shoot proliferation (90%). Maximum rooting 100% with 12.4 roots per explants were recorded on the medium containing 0.2 mg/l of IBA. The crude protein extract showed strong antibacterial activity against the tested strains.

1.2 Antibacterial Phytocompounds of *Andrographis paniculata*

Sule et al. [38], isolated two antibacterial compounds viz., 3-O-β-D-glucosyl-14-deoxyandrographolide and 14-deoxyandrographolide (Fig. 1 (a), (b)) from methanol extract of the whole plant of *Andrographis paniculata* against *Staphylococcus aureus, Streptococcus pyogenes, Micrococcus luteus, Proteus mirabilis* and *Pseudomonas aeruginosa* using cup-plate agar diffusion method. The structures of the compound were analyzed using spectroscopic methods (UV, IR, ^{1}H- and ^{13}C NMR). Results showed that the compound displayed significant antibacterial activities against the selected microbial strains.

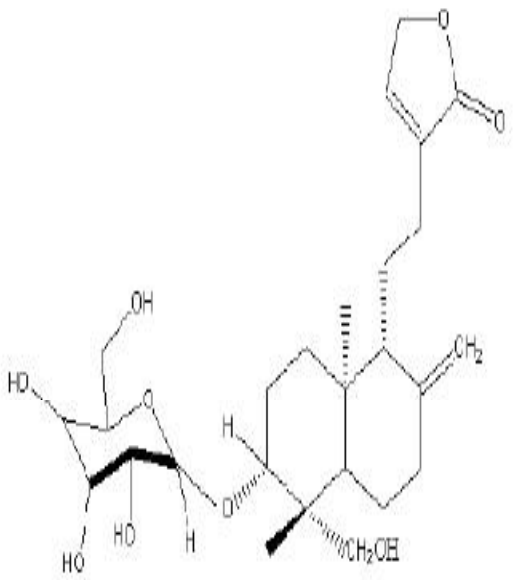
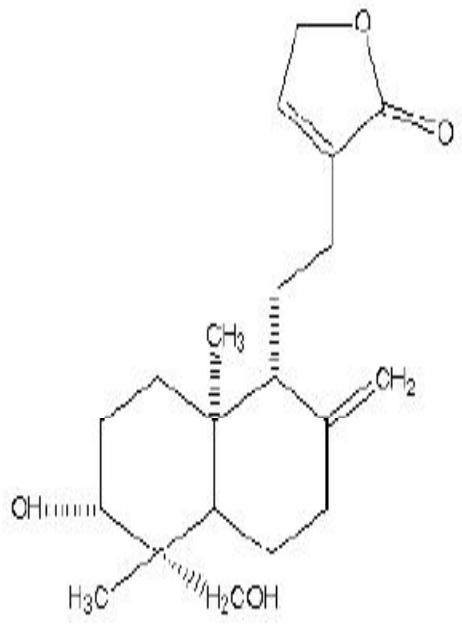


Fig. 1. (a) Structure of compound 1 (3-O-β-D-glucosyl-14-deoxyandrographolide)



(b) Structure of compound 2 (14-deoxyandrographolide)

Table 1. Summary of the review showing different studies done on antibacterial activities and phytocompounds of *Andrographis paniculata*

S. no.	Plant parts	Extracts	Test organisms effected	Phytochemical/phyto compound	Techniques/Instruments	References
1	Leaf	Hexane, chloroform	*Pseudomonas aeruginosa, Clostridium perfringens, Serratia marcescens, Bacillus subtilis, Enterobacter aerogenes, Shigella flexneri, Staphylococcus aureus* and *Salmonella typhi.*	Steroids/terpenoids, coumarin	Disc diffusion	Sukesh et al. 2011 [16]
2	Leaf, whole plant	Methanol, aqueous	*Staphylococcus aureus, Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris*	-	Agar well diffusion	Kumar et al. 2010 [17]
3	Whole plant	Ethanol	*Legionella pneumophila* and *Bordetella pertussis*	-	Disc diffusion method	Youhong et al. 2006 [18]
4	Root	Hexane, methanol	*Bacillus pumilus, Bacillus subtilis, Escherichia coli* and *Proteus vulgaris*	-	Agar well diffusion	Radhika et al. 2008 [19]
5	Whole plant	Dichloromethane, methanol, aqueous	*Staphylococcus saprophyticus, Staphylococcus epidermis, Staphylococcus aureus, Streptococcus pyogenes, Bacillus anthracis, Micrococcus luteus, Enterococcus faecalis, Proteus mirabilis, Proteus vulgaris, Klebsiella pneumoniae, Neisseria meningitis* and *Pseudomonas aeruginosa*	Terpenoids, tannins, saponins, flavonoids, alkaloids	Disc diffusion	Sule et al. 2010 [20]
6	Leaf	Ethanol	*Escherichia coli, Staphylococcus aureus and Micrococcus luteus*	-	Agar well diffusion	Doss and Kalaichelvan, 2012 [21]
7	Whole plant	Methanol	*Staphylococcus aureus, Streptococcus pyogenes, Micrococcus luteus, Proteus mirabilis* and *Pseudomonas aeruginosa*	3-O-β-D-glucosyl-14-deoxyandrographolide and 14-deoxyandrographolide	Agar well diffusion/UV, IR, ^{1}H- and ^{13}C NMR spectroscopy	Sule et al. 2011 [24]
8	Leaf, stem	Petroleum ether, acetone, chloroform, methanol	*Enterococcus faecalis, Streptococcus pyogenes, Klebsiella pneumonia* and *Proteus vulgaris*	Flavonoids, alkaloids	Disc diffusion	Radha et al. 2011 [23]
9	Whole plant	Dichloromethane, methanol, aqueous	*Staphylococcus saprophyticus, Staphylococcus epidermis, Staphylococcus aureus, Streptococcus pyogenes, Bacillus anthracis, Micrococcus luteus, Proteus mirabilis, Proteus vulgaris, Neisseria meningitis, Pseudomonas aeruginosa*	Terpenoids, flavonoids	Disc diffusion	Sule et al. 2011 [24]
10	Leaf, stem, root	Chloroform	*Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*	Carbohydrate, protein, flavonoid	Agar well diffusion	Kataky and Handique, 2010 [25]
11	Aerial part	Ethanol	*Escherichia coli* K 12 ROW, *Staphylococcus aureus* 29737, *Staphylococcus*	-	Disc	Mishra et al.

S. no.	Plant parts	Extracts	Test organisms effected	Phytochemical/phyto compound	Techniques/Instruments	References
			aureus ML 59, *Shigella boydii* 8, *Salmonella typhimurium* NCTC 74, *Shigella sonnei* 2, *Vibrio cholerae* 854, *Vibrio cholerae* 811, *Salmonella typhi* 59, *Vibrio alginolyteus* and *Bacillus licheniformis* 10341		Diffusion	2009 [26]
12	Leaf	Ethanol, methanol	*Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus vulgaris*.	-	Agar well diffusion	Anitha et al. 2013 [27]
13	Leaf	Acetone, ethanol	*Staphylococcus aureus, Bacillus subtilis*	Saponins, flavonoids	Disc diffusion	Hosamani et al. 2011 [28]
14	Flower	Acetone, ethanol	*Staphylococcus aureus, Bacillus subtilis*	-	Disc diffusion	Suneetha and Ravi [29]
15	Leaf	Ethanol	*Proteus vulgaris, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli*	-	Agar cup plate	Mishra et al. 2013 [30]
16	Aerial part	Chloroform	*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhimurium, Enterobacter cloacae*, *Staphylococcus aureus, Bacillus subtilis, Enterobacter faecalis* and *Staphylococcus epidermidis*.	Phenols, aromatic carboxylic acids and esters	Agar well diffusion, GC-MS	Roy et al. 2010 [31]
17	Root, stem	Chloroform	*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Proteus vulgaris*	-	Agar cup plate	Parvataneni and Koduru, 2010 [32]
18	Whole plant	Hexane, chloroform, methanol	*Erwinia caratovora*, *Pseudomonas marginales, Pseudomonas syringae, Pseudomonas aeruginosa and Xanthomonas compestris*	-	Agar well diffusion	Bobbarala et al. 2009 [33]
19	Leaf, root	Chloroform	*Staphylococcus aureus*	-	Agar well diffusion	Sivananthan and Elamaran, 2013 [34]
20	Leaf, stem, branches	Methanol, aqueous	*Bacillus subtilis, Salmonella typhi, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Enterobacter faecalis* and *Pseudomonas aeruginosa*	-	Agar well diffusion	Arunadevi et al. 2010 [35]
21	Leaf	water, methanol, ethanol, chloroform	*Bacillus cereus and Staphylococcus aureus, Escherichia coli and Salmonella enterica,*	Alkaloids, Saponins, Flavanoids, Tannins, Terpenoids, Steroids	Disc diffusion	Malahubban et al. 2013 [36]
22	Leaf	-	*Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus, Bacillus subtilis* and *Mycobacterium smegmatis*	-	Disc diffusion	Mamun et al. 2014 [37]

2. CONCLUSION

Medicinal plants are economically very essential, as they contain active constituents that are used in the treatment of many human diseases. From the above review and Table 1 above, it is clear that there were many studies which shows antibacterial activities of *Andrographis paniculata,* but a very few has been found on characterization of its phytocompounds. Many antibacterial works on *Andrographis paniculata* were found against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis,* which shows the effectiveness of the plant towards these strains and treatment of diseases caused by these bacteria. Leaves were found to be the most promising part to isolate an antibacterial compounds. Also ethanol, chloroform and methanol were came out to be the most promising solvents to extract phytocompounds showing broad spectrum antibacterial properties. As the plant is considered a potential source of antibacterial agent to develop new antibiotics, so there is an urgent need to isolate novel compounds from it so as to make a healthy tomorrow.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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