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Determination of Antimicrobial Activity and Phytochemical Screening of Selected Medicinal Plants in Tigray region of Northern Ethiopia

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ABSTRACT

Present paper evaluates the antimicrobial activities and preliminary phytochemical screening of the extracts of *Beciumgrandiflorum, Meriandrabengalensis, Tamarindusindica, Balanitesaegyptiaca* and *Otostegiaintegrifolia*. Comparative study was made for the selected traditional medicinal plants. The results of phytochemical test indicate that the bioactive chemical components show presence of alkaloids, flavonoids, phenols, carbohydrates, saponins and terpenoids. The antimicrobial activities of aqueous and ethanol extracts of leaf and stem were determined on the selected bacteria strains. The results indicate that the aqueous and ethanol leaf extracts of *Meriandrabengalensis* show better antimicrobial activity against *Staphylococcus aureus* and *Staphylococcus pneumonia* with inhibition zone diameters of 15.97 ± 0.09 and 19.03 ± 0.15 mm respectively, significant at ($P<0.05$) compared to other extracts. From the phytochemical components tested, carbohydrate was present in *Becium grandiflorum* extract. The lowest inhibition zone (4.5 ± 0.29 mm) was achieved for the extract of *Balanitesaegyptiaca.* The phytochemical component saponinis significantly present in *Meriandrabengalensis* in the aqueous leaf extract. In the comparative study made for the extraction solvents aqueous and ethanol, the aqueous extraction had strong inhibition zone. Therefore, a study need to be done on the isolation, identification, and quantitative determination of antimicrobial components present in the *Meriandrabengalensis* for its application in both animal and human pharmaceutical industries.

Keywords: Antimicrobial activities; Phytochemical screening; Extracts; Inhibition zone; Medicinal plants; Tigray; Ethiopia.

1. INTRODUCTION

Traditional medicinal plants are used by the local people to cure human and animal ailments around their localities. The traditional healers do not know the dose of the plants to heal the diseases caused by different pathogens. There are some documented traditionally (Gidey, 2010) used medicinal plants in Tigray region, northern Ethiopia but there is no documented research on

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the antimicrobial activities of the traditionally used medicinal plants.Different plant species yield a wide spectrum of chemical complexes with apparently no direct contribution to their growth and development, which are known as secondary metabolites. They are divided in to three major groups terpenes, nitrogen-containing, and phenolic compounds with various biological properties in plants that are used for a wide variety of diseases including cancers (Shirzad et al., 2011; Azadmehr et al., 2011), neurological disorders (Rabiei et al., 2014), chronic inflammation and lesions, particularly due to diabetes (Baradaran et al., 2013; Behradmanesh et al.,2013), atherosclerosis (Madihi et al., 2013; Setorki et al.,2011), cardiovascular diseases (Khosravi-Boroujeni et al., 2013; Sadeghi et al., 2014), and wounds (Asadi et al., 2013).Numerous plants containing volatile oils, polyphenols and alkaloids as active constituents are utilized as widespread folk medicines, while others gained popularity in the form of finished products collectively named phytomedicines (Kaushik and Goyal, 2008).

During the second half of the 20th century, taking traditional medicine as a substitute form of health care and the development of microbial resistance to the classical antibiotics led researchers to investigate the antimicrobial activities of medicinal plants. Antimicrobials of plant origin have enormous healing potential (Cowan, 1999). One of the vital activities possessed by these traditionally used medicinal plants is antimicrobial. The scarcity of infective diseases in plants is in itself signal of the successful defense mechanisms developed by them (Bolla et al., 2011). The substances that can either inhibit the growth of bacteria or kill them, with no toxicity or minimum toxicity to host cells are considered entrants for developing new antimicrobial drugs (Lee et al., 1998). Some of the bioactive compounds could deter the life processes of disease-causing bacteria, either by itself or in combination with other therapeutic agents (Sivananthan, 2013). In recent years, antimicrobial properties of medicinal plants are being progressively reported from different parts of the world (Grosvenor et al., 1995; Ahmad and Beg, 2001; Abu-Shanab et al., 2008). It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However, very little information is available on such activity of medicinal plants (Lee et al., 1998).

In third world countries, medicinal plants serve as alternative resources for health care of human diseases and animal ailments (Alexiadesand Lacaze, 1996; Lozoya, 1996; Robineau and Soejarto, 1996). Most of the world population depends on the natural traditional medicinal

plants. Different kinds of medicinal plants have active biochemical components that can inhibit growth of pathogenic organisms (Chitravadivu et al., 2009). The number of diseases that infect human not only increasing throughout the world (Gupta et al., 2004) but also the frequency of life threatening infection caused by pathogenic organisms is also increasing worldwide. In the last decade, research related to medicinal plants has been a burning issue for biological science disciplines (Pennaet al., 2003). Medicinal plants are known to be the main health care sources for large number of people throughout the world (Reddy and Jose, 2010).Traditional medicinal plants and their-derived medicines are widely used in old-style cultures in most of the world population, and are also becoming progressively common in modern societies as natural options to synthetic chemicals. About 70-80% of the rural population in many tropical countries depends on traditional medicines for their health care (Farnsworth et al., 1985).

Modern antibiotics are at times related with side effects whereas it is minimum or insignificantin using antimicrobial component of medicinal plants. Though, there can be smaller amount side effects, but have better tolerance and somewhat less expensive (Bari et al., 2010). Antibacterial components of medicinal plants and their use to cure the microbial illness as potential alternatives to artificial drugs to which many infectious microorganisms have become resistant seem to be very much promising (Singh et al., 2010). However, several studies have indicated that medicinal plants contain bioactive components e.g. peptides, unsaturated fatty acid, aldehydes, flavonoids, alkaloids, essential oils, phenols and water or ethanol soluble compounds. These secondary metabolites produced by plants are organic chemicals of high structural density which play diverse functions including chemotherapeutic, bactericidal, bacteriostatic and antimicrobial functions (Samuelsson, 2004).

Plants have always formed an important component in providing of primary healthcare in many communities in Africa. The HIV and AIDs pandemic, increase of resistance by pathogens to modern drugs (Susana et al., 2007), coupled with the rise in the number of immuno-compromised individuals suffering from opportunistic infections necessitates a search for alternative cure for these life threatening scourge. Therefore, the medicinal plants provide an alternative for potential cure for these challenges. In recent years, the studies on the plants having different values in their medicinal properties have been evaluated for possible antimicrobial activity and potential cures for a variety of ailments especially of microbial origin

(Kesavan,2007). There is no or limited research study conducted on the medicinal plants in Tigray region of northern Ethiopia. A study was therefore undertaken to assess the presence of the secondary biological components in the traditionally used selective medicinal plants present in Tigray region so that it will serve as starting point for researchers who need to conduct related work activities. The aim of the study was three fold, i)to measure the inhibitory activity of plant species,*Beciumgrandiflorum, Meriandrabengalensis, Tamarindusindica, Balanitesaegyptiaca* and *Otostegiaintegrifolia* extracts on *Staphylococcus aureus* and *Staphylococcus pneumonia;* ii)to screen out phytochemical (the bioactive ingredients) of the plants extracts; and iii) to assess inhibitory activity of each of the traditional medicinal plant extracts.

1.1. Description of the Study Area

The study was conducted in Mekelle University, Mekelle, capital city of the Tigray regional state of northern Ethiopia. The areas around Mekelle city are selected for medicinal plants collection. Mekelle is located about 780 kilometers north of Addis Ababa,Ethiopian capital, geographically lies at 13°29'N latitude, 39°28'E longitude, with an elevation of 2084 meters above mean sea level. The agro- ecological condition of the study areas around Mekelle is almostmiddle highland. Among themedicinal plants identified in different areas around Mekelle, the medicinal plant species that are found in almost all agro-ecological ecosystems were chosen for the study. Details are given in tables 1 to 3.

2. METHODOLOGY

2.1. Selection of the Traditional Medicinal Plants

The medicinal plants were selected from different areas around Mekelle based on informants' consensus factor (ICF). This was performed by gathering information about the traditional use of the medicinal plants from local informants. First, some traditional medicinal plants were selected based on the local healers. Then, seven informants asked on which medicinal plant cures for a given disease so that the more repeatedly chosen plant will be selected. Based on the informants' consensus factor method (Martin, 1995) the traditionally used medicinal plants were collected.The medicinal plants reported by five and more informants (≥17%) around the areas where the plants are collected fromthe surveyed local areas of the study (Table 1). A total of around 51 informants were participated during the assessment of the traditionally used medicinal

plants. The informants were consulted about the traditionally used medicinal plantsand the first selected by ten and more informants were examined for their antimicrobial activities and phytochemical screening test.Out of which, top five selected by 33% and more were considered for the laboratory extraction test activities. The five tested traditionally used medicinal plants are given in table 2.

Table 1. List of traditional medicinal plantspresent in areas around Mekelle.

Species	***Number of informants reporting about the medicinal value of the medicinal plants***	***Percentage (%)***
Becium grandiflorum	15	50
Meriandrabengalensis	15	50
Tamarindusindica	13	43
Balanitesaegyptiaca	12	40
Otostegiaintegrifolia	10	33
Rumexnervosus	7	23
Croton macrostacyus	6	20
Premnaschimperi	5	17
Carica papaya	5	17
Verbena officinalis	5	17

Table 2. Traditional use of themedicinal plants by the local people.

Botanical name	***Family name***	***Local name***	***Traditional use***
Beciumgrandiflorum	Lamiaceae	Tebeb	Antimalarial (locally, tseretantu)
Meriandrabengalensis	Lamiaceae	Mesaguh	Animal ailment (local name, afomar)
Tamarindusindica	Fabaceae	Humer	Anti-diarrhea (locally tseretekmat)
Balanitesaegyptiaca	Balanitaceae	Meqi	Anti-diarrhea (locally tseretekmat)
Otostegiaintegrifolia	Lamiaceae	Chindog	Animal ailment (locallyhmamkunchi)

Table 3. Collection sites of the medicinal plant materials around Mekelle.

Botanical name	***Place of collection***	***Elevation (m)***	***Latitude***	***Longitude***	***Month/Year***
Beciumgrandiflorum	Ambalaje	2490	12Â°56N	39Â°31E	March 2014
Meriandrabengalensis	Adigudem	2100	13Â°15N	39Â°31E	December 2014
Tamarindusindica	AbiAddi	1917-2275	13Â°3723"N	39Â°0006"E	February 2014
Balanitesaegyptiaca	AbiAddi	1917-2275	13Â°3723"N	39Â°0006"E	February 2014
Otostegiaintegrifolia	Mekelle	2084	13Â°29N	39Â°28E	January 2014

The information documented in table 2 is obtained from the local informants of the traditional medicinal plant species collected from different places around Mekelle (Table 3). The local traditional healers know the indigenous medicinal plants by transferring information from generation to generation orally.

2.2. Collection and Preparation of Plant Materials

The fresh plant parts samples were rinsed thoroughly to remove unnecessary materials two to three times using distilled water, cut into small pieces with sterile blade and dried in a cool dry area for about 7 to 10 days (25 -30^{o}C). While drying the plant parts were out of reach of sunlight in order to prevent loss of the bioactive components. The dried plant parts were crushed to fine powder and stored in air tight dark bottles at room temperature. Ten gram of each plant parts was extracted by mixing with distilled water (100 ml) influx for 48 hours at room temperature. Extracts were filtered through Watman No.1 paper. All the extracts were evaporated in rotary evaporator (Laborota-4000-efficient made in Germany) material in Mekelle University Organic chemistry laboratory. The alcoholic extracts were concentrated in a soxhlet (made in UK) apparatus at 60^{o}C for 30 min and aqueous extracts were freeze dried. All extracts were stored at 4^{o}C until further analysis (Wijeratne et al., 2006; Hatamnia et al., 2013).

2.3. Microorganisms

The clinical isolates of *Staphylococcus aureus and Staphylococcus pneumonia* used in the study were obtained from College of Veterinary Medicine, Department of Microbiology, and MekelleUniversity. These isolates were identified on the basis of morphological, cultural and biochemical characteristics (Collee and Marr, 1996).

2.4. Qualitative Phytochemical Screening of the Plants

To test the qualitative screening of the phytochemicals of the medicinal plants the procedure of Adedeji et al. (2012) was followed.

2.5. Statistical Data Analysis

All results of the measurements were done in triplicates to test the reproducibility of the experimental data. SPSS 16 (One Way ANOVAs) was used for statistical analysis of the results. All results are presented as mean ± Standard Error of mean. The values of $P<0.05$ were considered statistically significant.

3. RESULTS

3.1. Evaluation of Antimicrobial Activities of Plant Extracts

As shown in table 4, there is formation of clear zone that indicates the medicinal plants have inhibition zones of different sizes. The Maximum Inhibition Concentration (MIC) and Minimum Inhibition Concentration (MIC) of the extracts were 10 µl/disc and 5µl/disc respectively. All the experimental results obtained are the average of triplicates.

Figure 1. Preparation of the crude extracts in the laboratory.

Table 4. Antibacterial activity of aqueous leaf extracts against pathogenic organisms.

Medicinal Plant Species	***Parts used***	***Test Organisms***		
		Staphylococcus aureus	***Staphylococcus pneumonia***	
Beciumgrandiflorum	Leaf	13.53±0.32	17.07±0.18	Inhibition zones (mm)
	Stem	11.17±0.18	13.23±0.28	
	Root	10.17±0.88	12.43±0.23	
Meriandrabengalensis	Leaf	15.97±0.09	19.03±0.15	
	Stem	11.17± 0.2	14.1±0.15	
	Root	9.63±0.22	10.6±0.23	
Tamarindusindica	Leaf	12.03±0.15	13.13±0.19	
	Seed	12.5±0.25	13.77±0.15	
	Bark	9.73±0.19	10.73±0.13	
Balanitesaegyptiaca	Leaf	6.5±0.29	6.83±0.22	
	Stem	4.5±0.29	6.33±0.22	
	Bark	6.43±0.23	7.03±0.12	
Otostegiaintegrifolia	Leaf	6.23±0.27	6.1±0.3	
	Stem	6.43±0.27	7.1±0.3	
	Root	7.73±0.15	8.37±0.09	
Amoxicillin(5mg/ml)		23.03±0.88	24.03±0.09	

Note: Data is expressed as Mean zone of inhibition in millimeter ± SE(Standard Error).

To observe the qualitative test of biologically active components of the plants extracts,leaves, barks, stems, roots and seeds for some was subjected to be dried in air without exposing to full sun light in order to prevent loosing of some volatile components from the parts of the plants. The plant part materials were crushed using metal grinding equipment to increase surface area to volume ratio. The powder of the plant materials was subjected to the solvents of ethanol and water in flasks over 48 hours and filtered using Watman No.1 paper as indicated in figure 1. Then, the filtrate was subjected for farther crude oil extraction in the laboratory using soxhlet evaporator.

In the antibacterial activities of aqueous leaf extracts of the medicinal plant *Meriandrabengalensis* inhibited growth of *Staphylococcus pneumonia* with 19.03 mm zone which is maximum inhibition zone when compared to the growth of *Staphylococcus aureus* which is 15.97mm (Table 4). The reason for this might be that the crude extracts of the plant species *Meriandrabengalensis* could be reactive with the organic solvent ethanol so that less amount of the crude extracts have low action on the organisms.

Table 5. Comparative study of ethanol leaf and stem extracts against *Staphylococcus aureus.*

Medicinal Plant Species	***Parts used***	***Inhibition zone (mm)***
Becium grandiflorum	Ethanol leaf extracts	10.2±0.12
	Ethanol stem extracts	10.23±0.18
	Mixture of leaf and stem (1:1)	12.47±0.29
Meriandrabengalensis	Ethanol leaf extracts	12.27±0.18
	Ethanol stem extracts	11.3±0.18
	Mixture of leaf and stem (1:1)	14.87±0.19
Balanitesaegyptiaca	Ethanol leaf extracts	5.43±0.35
	Ethanol stem extracts	5.13±0.19
	Mixture of leaf and stem (1:1)	8.03±0.32
Otostegiaintegrifolia	Ethanol leaf extracts	5.77±0.26
	Ethanol stem extracts	6.03±0.39
	Mixture of leaf and stem (1:1)	7.1±0.29

Note*:* Data is expressed as Mean zone of inhibition in millimeter ±SE (Standard Error).

When the leaf extracts of *Meriandrabengalensis* using water and ethanolare compared, the water extracts of the medicinal plant have higher inhibition zone than that of ethanol extracts against growth of the bacterial species*Staphylococcus aureus* (Tables 4 and 5). The 1:1 ratio of the mixtures of the extracts of the plant parts had higher zone of inhibition when compared with their

single activities. This may be due to the activities of both mixed parts of the extracts might have supplementary activities to inhibit growth of the microbial organisms.

3.2. Phytochemical Screening of the Plants

Qualitative Phytochemical screening test was performed using standard procedures (Edeoga et al., 2005; and Adedeji et al., 2012). Of the bioactive components screened from the plants carbohydrate was observed highly in the species *Beciumgrandiflorum* (leaf) and the component saponins was highest in the plant extract of *Meriandrabengalensis* (leaf). Other components like Alkaloids, Flavonoids and Phenols are not clearly observed, may be due to the method/procedure followed is not accurately implemented or there may be some technical problems (table 6).

Table 6. Tested bioactive components of water extracts of medicinal plants.

Medicinal Plant Species	***Tested Phytochemicals***					
	Alkaloids	***Flavonoids***	***Phenols***	***Carbohydrate***	***Saponins***	***Terpenoids***
Becium grandiflorum(leaf)	-	-	-	++	+	+
Meriandrabengalensis(leaf)	-	-	-	+	++	+
Tamarindusindica(seed)	-	-	-	+	+	+
Balanitesaegyptiaca(fruit)	-	-	-	+	+	+
Otostegiaintegrifolia(leaf)	-	-	-	+	+	+

Note: ++ = strong presence, **+** = moderate presence, **-** = absence.

Table 7. Tested bioactive components of ethanol extracts of medicinal plants.

Medicinal Plant Species	***Tested Phytochemicals***					
	Alkaloids	***Flavonoids***	***Phenols***	***Carbohydrate***	***Saponins***	***Terpenoids***
Becium grandiflorum(leaf)	+	-	-	-	-	-
Meriandrabengalensis(leaf)	++	-	-	-	-	-
Tamarindusindica(seed)	+	-	-	-	-	-
Balanitesaegyptiaca(fruit)	+	-	-	-	-	-
Otostegiaintegrifolia(leaf)	+	-	-	-	-	-

Note: ++ = strong presence, **+** = moderate presence, **-** = absence.

Table 8. Tested bioactive components of methanol extracts of medicinal plants.

Medicinal Plant Species	***Tested Phytochemicals***					
	Alkaloids	***Flavonoids***	***Phenols***	***Carbohydrate***	***Saponins***	***Terpenoids***
Beciumgrandiflorum(leaf)	-	-	-	-	+	+
Meriandrabengalensis(leaf)	-	-	-	-	-	++
Tamarindusindica(seed)	-	-	-	-	-	+
Balanitesaegyptiaca(fruit)	+	-	-	-	-	+
Otostegiaintegrifolia(leaf)	-	-	-	-	-	+

Note: ++ = strong presence, **+** = moderate presence, **-** = absence.

Table 9. Tested bioactive components of acetone extracts of medicinal plants.

Medicinal Plant Species	***Tested Phytochemicals***					
	Alkaloids	***Flavonoids***	***Phenols***	***Carbohydrate***	***Saponins***	***Terpenoids***
Beciumgrandiflorum(leaf)	-	+	+	-	-	-
Meriandrabengalensis(leaf)	-	+	+	-	-	-
Tamarindusindica(seed)	-	+	+	-	-	-
Balanitesaegyptiaca(fruit)	-	-	+	-	-	-
Otostegiaintegrifolia(leaf)	-	-	+	-	-	-

Note: **+** = moderate presence, **-** = absence.

4. DISCUSSION

Ethanol used in the present study as an organic solvent to assess the antimicrobial activities of the traditionally used medicinal plants is commonly used solvent to test ethanol extracts of leaf of medicinal plants against *Staphylococcus aureus* bacterial species(Demetrio et al., 2015). Data on the traditional use of the medicinal plants is collected by the local people (Tables 1 and 2) during field work using consensus informant factor method. During the survey, different informants indicated the same plants and same usesespecially for some widely used medicinal plants. The informant consensus is helpful tosee the similarity of information given by the informants to confirm the authenticity ofinformation by comparing it with other information given by other informants on thesame medicinal plant. Accordingly, diseases of humans, numbers of citations and percentages arerecorded. The tested traditional medicinal plants were selected following a standard manual. The Informant Consensus Factor (ICF) is followed based on Martin (1995). Of the bioactive components screened from the plants, carbohydrate was observed highly in the species *Beciumgrandiflorum* (leaf) and the component saponin was highest in the plant extract of *Meriandrabengalensis* (leaf). The other components like alkaloids, flavonoids and phenols were not clearly observed. This may be due to the method/procedure that is followed might not be accurately implemented. There might be some technical problems (Table 5).

The results for the antimicrobial activities were observed after applying 10 µL/disc of the extracts oil which completely inhibited up to diameters of 4.5-19.03 mm. Furthermore, the minimum inhibitory concentration (MIC) was estimated to be lower than 5 µL /disc. Some plants contain many biologically active compounds which are widely used to meet certain primary healthcare needs especially in most rural communities. They could serve as sources of lead

compounds for the development of putative antimicrobial agents especially now that some synthetic drugs are barely efficacious against some pathogenic bacteria.

In the present case, the aqueous leaf, stem and bark extracts of *Beciumgrandiflorum, Meriandrabengalensis, Tamarindusindica, Balanitesaegyptiaca* and *Otostegiaintegrifolia* were investigated against some selected pathogenic bacteria. The results indicate that the aqueous and ethanol leaf extracts of *Meriandrabengalensis* show strong antimicrobial activity against *Staphylococcus aureus* and *Staphylococcus pneumonia* with inhibition zone diameters of 15.97 ± 0.09 and 19.03 ± 0.15 mm (Table 4) respectively, significant at $P<0.05$ compared to the other tested extracts. Only aqueous extracts of the plants were used on antimicrobial activities. In the comparative study indicated in table 4 only antimicrobial activity against the bacterial strain of *Staphylococcus aureus* was made. The reason for this might be theparticular bacterial strain has low inhibition zone when compared to the other bacterial strain, *Staphylococcus pneumonia.* In the comparative study of ethanol leaf and stem extracts against *Staphylococcus aureus* of *Meriandrabengalensis,* the bacterial strain had better resistance to the extracts. This might be due to the properties of the solvent used (Table 5).

The maximum inhibition zone of aqueous extract of leaf of *Meriandrabengalensis* against the bacterial species of *Staphylococcus pneumonia* is 19.03 mm. The minimum inhibition zone of aqueous extract of *Balanitesaegyptiaca* stem is 4.5mm. The reason for this lower inhibition zone is considered to be due to the resistance of the bacterial species.

Moreover, in comparative study of ethanol leaf and stem extracts against *Staphylococcus aureus* all the extracts have lower inhibition zones. Carbohydrate, saponins and terpenoids were present in the aqueous extracts of all the tested traditional medicinal plants. The evidence for the sign ++ (double positive) was the results obtained when observed manually. Because the phytochemical screening was done following the standard procedure, the results indicated were observed manually and expressed aspositive, negative and double positive (Tables 6 to 9).

The qualitative test of biologically active components of the medicinal plants was done following a standard manual (Kensa et al., 2011).The organic solvent methanol was also used as a solvent to observe presence of the biological component/compounds (Demetrio et al., 2015). The methanol extract of *Meriandrabengalensis* (leaf) was observed easily for the presence of terpenoids. The other medicinal plants were also observed for the presence of terpenoids whereas

other components alkaloids, flavonoids, phenols and carbohydrate were not observed in all the traditionally used medicinal plant species.

5. CONCLUSION

From the study it can be concluded that all the traditionally used medicinal plants screened in this study have some phytochemicals in common while the antimicrobial activity is more with *Meriandrabengalensis* compared to other plants. The lowest antimicrobial activity was observed in *Balanitesaegyptiaca* extracts. However, there is a need to conduct detail study on the medicinal plants with regard to selection of test organisms (bacterial and fungal strains) using different organic solvents. Moreover, the study needs to be focused on isolation, identification, and quantitative determination of antimicrobial components present in the *Meriandrabengalensis* for its application in both animal and human pharmaceutical industries.There is a need to conduct detailed study on the number of ethno -botanically documented and traditionally used medicinal plants. This will facilitate and lead to the development of drug by pharmaceutical research institutes and companies.

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