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Antimicrobial activities of the leaves and roots of Elaeagnus umbellata Thunb

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The leaves and root of *Elaeagnus umbellata* (Elaeagnaceae) were extracted successively with various organic solvents and water. These crude extracts were evaluated for antimicrobial activities against three gram positive bacteria, five gram negative bacteria, one yeast and one fungus by using disc diffusion method. The acetone, petroleum ether, ethyl acetate and methanol extracts of leaves and roots of the plant exhibited prominent activities while chloroform, ethanol extracts showed moderate activity and water extract showed no activity against all the tested bacteria. Ethanolic and methanolic extracts also showed considerable activity against fungus and yeast. The root extracts of the plant were found more active against the microorganisms.

Key words: *E. umbellata*, extracts, fungi, yeast, antibiotic discs.

INTRODUCTION

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near East, but it is doubtless an art as old as mankind. Many workers have documented the traditional knowledge about the plants and their conservation. The traditional use of plants for curing various diseases and health problems is one of the major utilities. (Zafer 1996, Mhaskar et al. 2000, Behl and Sirivastava 2002 and Sharma 2003). The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Thus, any phytochemical investigation of a given plant will reveal a spectrum of its constituents. Historically, pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random

screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics (Gerhartz et al. 1985, Kroschwitz, and Howe-Grant, 1992). Even now, contrary to common belief, drugs from higher plants continue to occupy an important niche in modern medicine. On a global basis, at least 130 drugs, all single chemical entities extracted from higher plants or modified further synthetically, are currently in use, though some of them are now being made synthetically for economic reasons (Newman et al., 2000). Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava et al., 1996). A wide range of medicinal plant parts is used for extracts as raw drugs and they possess various medicinal properties. The different part used includes the root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local use, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries (Uniyal et al., 2006). Although hundreds

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of plant species have been tested for antimicrobial properties, the vast majority of them have not been adequately evaluated (Balandrin et al. 1985). Medicinal plants have been relied upon by 80% of the world population for their basic health care needs. Pakistan is no exception, as it has a variety of plants of medicinal importance (Tareen et al., 2002). The herbs extensively used for treating diseases, however their commercial exploitation is limited due to the lack of a scientific knowledge for their use (Ahmad et al., 2003). Among these plants, Elaeagnus umbellata Thunb, also called cardinal olive, autumn olive or autumn Elaeagnus (Dirr, 1983), a wild shrub belonging to the family Elaeagnaceae, is native to China, Japan and Korea, and is also found in Afghanistan and India (Potter, 1995) .The plant was introduced to the US in the 1830s from East Asia as an ornamental plant (Dirr. 1983). E. umbellata is widely distributed at a height of 4500-6000 feet above sea level in Azad Kashmir. It is abundantly found in Himalayan regions of Pakistan (Hensley, 1984, Ahmad et al., 2005). The E. umbellata is a large spreading, spinybranched shrub often obtaining 3.5-5.5 m in height, and 3.5-5.5 mm in width. The foliage is light green on top and a silvery green on the bottom. Leaves are alternate and petiolated in small lateral clusters on twigs (Eckardt, 1987). The fruit / berries are silvery with brown scales when immature and ripen to a speckled red in September - October (Sternberg, 1982). Its berry is an excellent source of vitamins A, C, E, flavonoids, essential fatty acids (Chopra et al., 1986), lycopene, carotene, lutein, phytofluene and phytoene. The lycopene content of the E. umbellata fruit is 17 times greater than that of tomato (Kohlmeier et al., 1997, Fordham et al., 2001). Many studies have proved that lycopene protects against myocardial infarction (Kohlmeier et al., 1997) and various forms of cancers including prostate cancer (Clinton, 1998, Giovannucci et al., 1996). The seeds of the plant are used as a stimulant in the treatment of coughs and seed oil is used in the treatment of pulmonary affections (Chopra et al., 1986). Various phytochemicals including palmitic acid (16.9%), eugenol (11.1%), methyl palmitate (10.5%), 4-methyl anisole (33-42.7%) and 4-methyl phenol (10.9-13.3%) have been isolated from the flowers of the plant (Matthews, 1994). The extracts of the plant and its chemical constituents exhibit antimicrobial properties, which may be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency (Nabeela and Zaheer, 2003). Many plants have been used due to their antimicrobial traits, which are due to the compounds synthesized in the secondary metabolism processes, that is, phenolics and tannins. E. umbellata is one of such plants which are being used against infectious diseases. Although antibacterial activity of the aerial parts of the plant had

been studied by Sabir et al., (2007) against four bacteria but a detailed antimicrobial potential of the aerial and under-ground parts of *E. umbellata* has not been studied, the *in vitro* antimicrobial activity of the leaves and roots of the plant growing wild in Azad Jammu and Kashmir was evaluated by using disc diffusion method against eight bacteria, one fungus and one yeast. The present work appears to be the first detailed antimicrobial bioassay report on aerial as well as ground part of the plant.

MATERIAL AND METHODS

Fresh plant parts were collected randomly from different localities of Muzaffarabad, Azad Jammu and Kashmir, Pakistan. The plant was identified by a Senior Botanist at the Department of Botany, where a voucher specimen (No. Bot. UAJK 1021) is deposited. The collected plant parts were separately dried carefully under shade and then homogenized to fine powder and finally stored in air tight bottles at 4°C.

Aqueous Extracts

50 grams of each ground plant part material was extracted with distilled water in soxhlet extraction apparatus (Thomas, 1977). These extracts were collected separately and each extract was dried on rotary evaporator under reduced pressure. The last traces of the water were evaporated at water bath, which was used as a source of heat (Rawlins and Tindall, 1977).

Organic Solvent Extraction

A portion (25g) of each dried powdered plant part was soaked separately in 250 ml petroleum ether, acetone, ethyl acetate, chloroform, ethanol and methanol. The extraction was carried out by maceration for 10 days in each solvent at room temperature (25±2 °C). The solvents, extracted material were filtered in flasks (Rawlins and Tindall, 1977). All extracts were then dried on a rotary evaporator, weighed and stored at 4°C until further analysis.

Preparation of Dilution

The dried aqueous, methanol, ethanol, petroleum ether, acetone, ethyl acetate and chloroform extracts were then dissolved in their respective solvents in a proportion of 10mg/ml. The concentration of reference antibiotics that is ciprofloxacin was 100 µg/ml and nystatin 1500 u/ml.

Micro organisms

In the present study, three gram-positive bacteria, Staphy-

Table1. Antimicrobial activity profile of the leaves of *Elaeagnus umbellata* Thunb.

Zones of inhibition (mm) ± standard error Mean (S E M)											
Sr. No.	Strains	AC	PE	EA	СН	ET	МТ	wT	CF	NS	
1	S. aureus	19.35±0.15	14.5±0.05	20.33±0.17	10.00±0.00	12.9±0.35	16.16±0.08	-	32.13±0.13	-	
2	B. subtilis	16.33±0.17	-	19.66±0.33	16.22±0.17	15.83±0.16	14.33±0.17	-	31.93±0.06	-	
3	E. faecium	-	-	-	-	13.9±0.1	17.33±0.19	-	30.03±0.03	-	
4	E. coli	19.22±0.11	16.11±0.10	22.96±0.23	20.56±0.23	17.46±0.33	16.33±0.16	-	31.83±0.17	-	
5	B. bronchisiptica	16.93±0.06	20.33±0.15	20.83±0.17	16.30±0.16	14.9±0.2	17.19±0.16	-	31.5±0.1	-	
6	P. aeruginosa	-	-	17.66±0.33	15.00±0.00	18.83±0.16	19.83±0.17	-	29.83±0.17	-	
7	P. syringae	-	-	22.66±0.33	17.16±0.16	15.66±0.33	20.93±0.06	-	30.83±0.17	-	
8	S. typhae	-	-	-	-	15.83±0.17	15.26±0.13	-	29.93±0.06	-	
9	S. cerevisiae	-	-	-	-	12.66±0.06	14.82±0.18	-	-	21.00±0.33	
10	A. flavus	-	-	-	-	10.23±0.1	09.33±0.17	-	-	15.00±0.26	

Key: AC: Acetone, PE: Petroleum ether, EA: Ethyl acetate, CH: Chloroform, ET: Ethanol, MT: Methanol, WT: Water, CP: Ciprofloxacin, NS: Nystatin; Concentration of crude extracts 100mg/ml, Ciprofloxacin 100µg/ml and Nystatin 1500u/ml

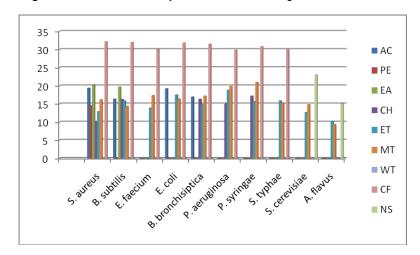


Figure 1. Antimicrobial activity of the leaves of Elaeagnus umbellata.

AC: Acetone, PE: Petroleum Ether, EA: Ethyl Acetate, CH:

Chloroform, ET: Ethanol.

MT: Methanol, WT: Water, CF: Ciprofloxacin, NS: Nystatin

lococcus aureus, Bacillus subtilis, Enterococcus faecium, five gram-negative bacteria, Escherichia coli, Bordetella Salmonella bronchisiptica, typhi, Pseudomonas aeruginosa, Pseudomonas syringae (local isolate), one veast Saccharomyces cerevisiae (local isolate) and one fungus Aspergillus flavus (local isolate), were used to check the antimicrobial potential of different extracts of the selected plant parts. The pure bacterial, fungal and yeast strains were obtained from the Department of Pathology Muzaffarabad Medical College Teaching Hospital, Muzaffarabad Azad Jammu and Kashmir. Bacterial strains were cultured overnight at 37°C in nutrient agar (NA, Oxoid, Hampshire, UK) while fungal strains were cultured overnight at 28°C sabouraud's dextrose agar (SDA, Oxoid, Hampshire, UK).

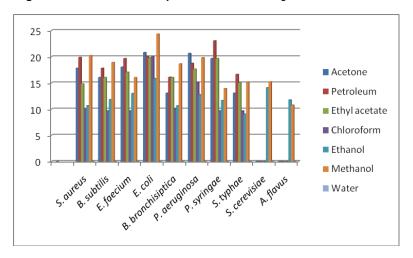
Antimicrobial Assay

The antimicrobial activity was determined by disc diffusion method (Vander and Vlientnck, 1991). Briefly, 100 µl of suspension of tested microorganisms, containing 10⁸ colony-forming units (cfu)/ml of bacteria cells and 10⁵ spores/ml of fungi was spread on sterilized nutrient agar (NA) and sabouraud's dextrose agar (SDA)

 Table 2. Antimicrobial activity of the Roots of Elaeagnus umbellata
 Thunb. Concentration of crude extracts 100mg/ml

Zone	Zones of inhibition (mm) ± standard error Mean (S E M)										
Sr. No.	Microorganisms	Acetone	Petroleum Ether	Ethyl acetate	Chloroform	Ethanol	Methanol	Water			
1	S. aureus	17.83±0.17	19.9±0.1	14.83±0.17	10.00±0.00	10.67±0.33	20.24±0.06	-			
2	B. subtilis	16.06±0.06	17.83±0.17	16.06±0.06	9.66±0.33	11.83±0.17	18.9±0.1	-			
3	E. faecium	18.06±0.06	19.66±0.33	17.06±0.06	9.66±0.33	13.00±0.00	16.03±0.3	-			
4	E. coli	20.83±0.17	20.06±0.06	19.83±0.17	20.06±0.06	15.83±0.16	24.33±0.17	-			
5	B. bronchisiptica	13.06±0.03	16.1±0.01	16.06±0.03	10.06±0.06	10.66±0.33	18.66±0.15	-			
6	P. aeruginosa	20.66±0.33	18.76±0.23	17.66±0.33	15.1±0.10	12.76±0.23	19.83±0.17	-			
7	P. syringae	19.66±0.33	23.06±0.06	19.66±0.33	9.66±0.33	11.66±0.33	13.93±0.16	-			
8	S. typhae	13.06±0.06	16.66±0.33	15.06±0.06	9.66±0.33	9.06±0.06	15.13±0.17	-			
9	S. cerevisiae	-	-	-	-	14.11±0.1	15.22±0.16	-			
10	A. flavus	-	-	-	-	11.75±0.15	10.77±0.13	-			

Figure 2. Antimicrobial activity of the Roots of Elaeagnus umbellata.



medium, respectively. The disc (6 mm in diameter) was individually impregnated with extract samples, placed on the agar plates which had previously been inoculated with the tested micro organisms. A disc without compound was used as a negative control. In the second series of experiment, antibiotic discs prepared from the dilution of commercially available standard reference antibiotics that is, ciprofloxacin and nystatin were placed on the top of the medium in the center of Petri dishes following the disc diffusion method (Vander and Vlientnck 1991). The purpose of this experimental set was to compare the antimicrobial activity of the standard reference antibiotics with that of the solvent extracts of leaves and roots of E. umbellata. Plates, after 2 h at 4°C, were incubated at 37°C for 24 h for bacteria and at 28°C for 72 h for fungal strains in incubator (Synou, Gemany). Antimicrobial activity was evaluated by measuring the diameter of the growth inhibition zones by zone reader

(MAS GmbH, Germany) in millimeters for the organisms and comparing to the controls (Rehman *et al.*, 2001).

STATISTICAL ANALYSIS

All values were expressed as mean ± standard error. The data for each micro organism were analyzed by using one way analysis of variance (ANOVA) technique and the mean were compared by using LSD at 5% (0.05) probability level (Steel and Torrie, 1980).

RESULT AND DISCUSSION

Plant extracts are being studied against bacteria for years and in a more intensified way in the last three decades. During this period, lots of antimicrobial screening evalua-

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tions has been published based on the traditional use of Chinese, African and Asian plant drugs (Forestiere et al., 1988). In the present study, the antimicrobial activities of acetone, petroleum ether, ethyl acetate, chloroform, ethanol, methanol and water extracts of E. umbellata leaves and roots were determined. The results of the antimicrobial screening of different solvents extracts of leaves and root of E. umbellata against 08 bacteria, 01 yeast and 01 fungus are presented in Table 1, figure 1 and table 2, figure 2. According to the previous investigations of Sabir et al., (2007) the samples prepared from the aerial parts of E. umbellata specie growing in Rawalakot Azad Jammu and Kashmir showed antimicrobial activity against four bacteria tested. They studied antibacterial activity of acetone, chloroform, methanol and ethanol extracts of leaves along with various extracts of berries and flowers against four bacteria namely E. coli, P. aeruginosa, S. aureus and B. subtilis. The present study showed 50 to 100% better results than the previous antibacterial activity. The acetone extracts of leaves showed antimicrobial activity against S. aureus, B. subtilis, E. coli, B. bronchisiptica, while root extract exhibited activity against S. aureus, B. subtilis, E. faecium, E. coli, B. bronchisiptica, P. aeruginosa, P. syringae, and S. typhae. The results are presented in Table 1, figure 1 and table 2, figure 2. The petroleum ether extract of the leaves of E. umbellata exhibited antimicrobial activities against S. aureus E. coli and B. bronchisiptic while root extract showed remarkable activity against S. aureus, B. subtilis, E. faecium, E. coli, B. bronchisiptica, P. aeruginosa, P. syringae and S. typhae. The results are presented in Table 1, figure 1 and table 2, figure 2. The ethyl acetate extract of the leaves of the plant showed antibacterial activity against S. aureus, B. subtilis, E. coli, B. bronchisiptica, P. aeruginosa, and P. syringae while the extract of root also showed promising activity against S. aureus, B. subtilis, E. faecium, E. coli, B. bronchisiptica, P. aeruginosa, P. syringe e, and S. tvphae (Table 1, figure 1 and table 2, figure 2). The chloroform extract of the leaves showed activity against Escherichia coli (20.56±0.23 mm), Pseudomonas aeruginosa (15.00 ±0.00 mm) Pseudomonas syringae (17.16 ±0.16 mm), Staphylococcus aureus, (10.00 ±0.00 mm) subtilis (16.22 ±0.08 mm), Bordetella bronchisiptica (16.30 ±0.16 mm), while Salmonella typhae, E. faecium, Saccharomyces cerevisiae and Aspergillus flavus were found inactive (Table 1, figure 1). The chloroform extract of the root of E. umbellata exhibited moderate activity against all the tested microorganisms used except Saccharomyces cerevisiae and Aspergillus flavus. The mean diameter of zones of inhibition of the extract Staphylococcus aureus, Bacillus subtilis, Enterococcus faecium, Escherichia coli, Bordetella bronchisiptica, Pseudomonas aeruginosa, Pseudomonas

syringa and Salmonella typhae were 10.00 ±0.00 mm, 9.66 ± 0.33 mm, 9.66 ± 0.33 mm, 20.06 ± 0.06 mm, 10.06 ± 0.06 mm, 15.1 ± 0.1 mm, 9.66 ± 0.33 mm and 9.66 ± 0.33 mm respectively (Table 2, figure 2). The chloroform extracts showed comparatively more in vitro antimicrobial activity against bacteria as compared to the previous work on leaf extract by Sabir et al., (2007) which may be due to topographic variation on the chemical constituents of the plant. The methanolic extracts of the leaves and root of the plant showed considerable activity not only against all tested bacteria but also exhibited considerable activity against the fungus and yeast. The zones of inhibition produced by the leaves extract against Enterococcus faecium, Pseudomonas aeruginosa. Pseudomonas syringae, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella typhae and Bordetella bronchisiptica were 17.33 ± 0.19 mm, 19.83 ± 0.17 mm, $20.93 \pm 0.06 \text{ mm}$, $16.16 \pm 0.08 \text{ mm}$, $16.33 \pm$ 0.16 mm ,15.26 \pm 0.13 mm and 17.19 \pm 0.16 mm, respectively. The zone of inhibition produced by the methanolic extract of leaves against S. cerevisiae and A. flavus were found to be 14.82 ± 0.18 mm, 09.33 ± 0.17 mm, respectively. The zones of inhibition produced by the methanolic extract of root against Enterococcus Pseudomonas aeruginosa, Pseudomonas Staphylococcus aureus, Bacillus subtilis, syringae, Escherichia coli, Salmonella typhae and Bordetella bronchisiptica were found to be 16.03 ± 0.3 mm, 19.83 ± 0.17 mm, $13.93 \pm 0.16 \text{ mm}$, $20.24 \pm 0.06 \text{ mm}$, 18.9 ± 0.1 mm, 24.33 ± 0.16 mm, 15.13 ± 0.17 mm and $18.66 \pm$ 0.15 mm, respectively. The zones of inhibition against Saccharomyces cerevisiae and Aspergillus flavus were 15.22 ± 0.16 mm and 10.77 ± 0.13 mm, respectively (Table 1, figure 1 and table 2, figure 2). Sabir et al., (2007) described that the ethanolic extract of the leaves of Elaeagnus umbellata did not show any significant antimicrobial activity against Pseudomonas aeruginosa, Staphylococcus aureus and E. coli except Bacillus subtilis. While in the present study it was observed that the ethanolic extracts of the leaves and roots were found to be more active against gram-positive and gramnegative bacteria as well as against fungus and yeast. The ethanolic extract of the leaves showed antimicrobial activity against Escherichia coli (17.46 ± 0.16 mm), Pseudomonas aeruginosa (18.83 ± 0.06) mm). (15.66 Pseudomonas syringae 0.33 mm), Staphylococcus aureus, (12.90 ±0.35 mm), Bacillus subtilis (15.83 ± 0.16 mm), Bordetella bronchisiptica (14.09 ±0.2 mm), Salmonella typhae (15.83 ±0.17 mm), Enterococcus faecium. (13.90 ±0.1 mm). Saccharomyces cerevisiae, (12.66 ±0.06 mm) and Aspergillus flavus, (10.23 ±0.1 mm). The ethanolic extract of the root of Elaeagnus umbellata also exhibited moderate activity against all the tested microorganisms including Saccharomyces cerevisiae and Aspergillus flavus. The

mean diameter of zones of inhibition of the extract against Staphylococcus aureus (10.67 ±0.33 mm),, Bacillus subtilis (11.83 ±0.17 mm), Enterococcus faecium (13.00 ±0.00 mm), Escherichia coli (15.83 ±0.16 mm), Bordetella bronchisiptica (10.66)±0.33 (12.76 Pseudomonas aeruginosa ±0.23 mm). Pseudomonas syringae (11.66 ±0.33 mm), Salmonella typhae (9.06 ±0.06 mm), Saccharomyces cerevisiae (14.11 ±0.1 mm) and Aspergillus flavus (11.75 ±0.15 mm), respectively. The detailed results are shown in Table 1, figure 1 and table 2, figure 2. The water extracts of the leaves and roots represented no activity against the microorganisms (Table 1, figure 1 and table 2, figure 2). The antibiotic, ciprofloxacin showed high activity against all the microorganisms used except Saccharomyces cerevisiae and Aspergillus flavus. The activity profile against Staphylococcus aureus, Bacillus Enterococcus faecium, Escherichia Bordetella bronchisiptica. Pseudomonas aeruginosa. Pseudomonas syringae. Salmonella typhae presented in Table 1, figure 1. The nystatin exhibited activities against the yeast and fungus while all the bacteria were resistant to nystatin (Table 1, figure 1). Avato et al., (1997) reported that extracts of Bellis perennis have a high antimicrobial activity against bacteria than fungus. The results of Zavala et al., (1997) were similar to ours. They showed that extracts from some plants have high activity against bacteria than yeast and fungus. On the other hand the antimicrobial activity against gram-negative bacteria was more effective than gram-positive bacteria (Table 1, figure 1 and table 2, figure 2). The antibacterial and antifungal activity of the plant extracts may be due to the flavonoids and phenolics which have already been reported in the leaves of the plant (Chopra et al., 1986). These compounds are usually extracted in organic solvents. Actually, phenolics not only attack the cell wall and cell membrane, thereby destroying its permeability and releasing the intracellular constituents (ribose, sodium, glutamate, and so forth) but also interfere with membrane function, for example, electron transport chain, nutrient uptake, protein and nucleic acid synthesis and also affect enzyme activity. The biactive compounds might have several invasive targets that could lead to inhibition of the bacteria and fungi (Sabir et al., 2007). The result revealed that extent of inhibition is variable for different extracts against different microbes. It seems very likely, therefore, that the antimicrobial compounds extracted from E. umbellata may inhibit bacteria and fungi by a different mechanism than that of currently used antibiotics and may have therapeutic value as an antimicrobial agent against multidrug resistant microbial strains. The method used by traditional healers for treating a bacterial and fungal infection is administering a decoction of the plant or by using a part by boiling it in

water. According to our results, an organic solvent extract is more effective in reducing microbial infections compared to water.

Conclusion

Plant based antimicrobials have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. The extracts of the leaves and roots of E. umbellata showed excellent antimicrobial activity against the tested bacteria, fungus and yeast. Therefore, it is concluded that the extract of the leaves and roots of the plant can be regarded as good natural antibiotics with considerable degree of antimicrobial activity and that they can be used in the treatment of various infectious diseases caused by resistant microorganisms. The results also revealed that the use of roots of E. umbellata may be more beneficial than the aerial parts against infectious diseases. Consequences, our further investigations are directed at isolation of pure compounds which are present in fractions showing large inhibitory activity against various microorganisms as well as other pharmacological or toxicological properties is aimed. Moreover, various parts of the plant may be used to treat various ailments as reported in the literature. Cultivation of this plant on commercial basis may also be employed so as to further increase the availability and to reduce the cost. Such usage may be more effective due to synergetic effect of various components rather than stand alone use of a pure compound.

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