**ABSTRACT**

The antibacterial activity of methanolic crude extract of *Diacure*, a polyherbal formulation was investigated against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Micrococcus luteus*, *Escherichia coli* and *Candida albicans* as test strains at different concentrations (80, 90 and 100 µg/disc). Better antibacterial activity was observed with the extracts of *Diacure*, that showed excellent inhibitory activity against *Klebsiella pneumoniae*, *Escherichia coli* and *Candida albicans* at concentration of 100 µg/disc. Among different bacteria tested *Klebsiella pneumoniae*, *Escherichia coli* and *Candida albicans* were found to be more sensitive to crude extract when compared to others. HPLC analysis of the crude extract of *Diacure*, a polyherbal formulation of 11 medicinal plants showed four different Phenolic acids (Tannic acid, Gallic acid, Ferulic acid and Caffeic acid). The results of the study provide scientific basis for the use of the plant extract in the future development as antioxidant, antibacterial, antifungal and anti-inflammatory agent.

**Keywords**

Antibacterial activity, HPLC, Phenolic acid, *Diacure*, Medicinal plants.
gently. After the plates were incubated at 35 ± 2°C for 48 hours, the inhibition zones around the discs where no growth occurred were measured in millimeters. The experiments were repeated in duplicate for all of the test strains.

**Sample Preparation of phenolic compounds**

The phenolic acids were extracted as per the method of Singh et al.17. One gram of each extract was macerated and suspended in 5 ml methanol-water (80:20; v/v). The collected samples were subjected to ultrasonication (Branson Sonifier, Danbury, CT, USA) for 15 min at 4°C followed by centrifugation at 12 500 x g for 15 min. The clear supernatant was subjected to charcoal treatment. The residue was re-extracted twice with the same extracting solution and the supernatant was pooled prior to evaporation under vacuum (Buchi Rotavapor Re Type, Labco, India; Ambala Cantt. India). Dried extract were resuspended in 1.0 ml high-performance liquid chromatography (HPLC)-grade methanol by vortexing and filtered through ultra membrane filter (pore size 0.45 µm: Millipore) before HPLC analysis.

**HPLC analysis**

Quantitative analysis of the sample was performed according to the method of Singh et al.17. The HPLC system (Shimadzu Corporation, Kyoto, Japan) was equipped with two Shimadzu LC-10 ATVP reciprocating pumps, a variable Shimadzu SPD-10 AVP UV-VIS detector and a Rhodyne Model 7725 injector with a loop size of 20 µl. The peak area was calculated with a Winchrom integrator. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250 x 4.6 mm i.d., particle size 5 µm, Luna 5 µ C-18(2); phenomenex, Torrance, CA, USA) at 25°C. Running conditions included: injection volume, 5µl; mobile phase, methanol: 0.4% acetic acid (80: 20 v/v); flow rate, 1 ml/min; and detection at 290 nm. Samples were filtered through an ultra membrane filter (pore size 0.45 µm; E-Merck, Darmstadt, Germany) prior to injection in the sample loop. Tannic, gallic, caffeic, ferulic, benzoic, cinnamic, capachin and salicylic acids were used as internal and external standards. Phenolic acids present in each sample were identified by comparing chromatographic peaks with the retention time (Rt) of individual standards and further confirmed by co-injection with isolated standards. The amount of each phenolic acid is expressed as micrograms per gram of fresh weight unless otherwise stated.

**Results and Discussion**

**Comparative analysis of antibacterial activity**

**HPLC analysis**

Recent researches indicate that the polyphenols, being secondary metabolites, are present in rich amount in several plants. Many of them possess antioxidant, anti-inflammatory and several others therapeutic properties. The HPLC fingerprints of the crude extracts of diacure showed four types of the Phenolic acids i.e. Tannic acid, gallic acid, Ferulic acid and caffeic acid that are present in varying amount (Fig. 2). Although a primary objective of carrying out HPLC may be to standardize dosage, more information may be obtained during the course of a run, if appropriate detection hardware and software are used.

**Results and discussion**

Table 1 provides the botanical name, family, plant parts used together mixed in 1:1 ratio forming a polyherbal formulation of 11 medicinal plants. Methanolic extract of diacure showed antimicrobial activity by inhibiting one or more microorganisms. The results of the antimicrobial screening of the methanolic extract were shown in figure 1 below. The tested plant extracts were most active against gram positive microorganisms than the gram negative microorganisms. This is in agreement with the previous reports by several workers (Gupta MP etal).

Methanol extracts of diacure, a polyherbal formulation were tested for antimicrobial activity with 80, 90 and 100µl/disc concentrations. The antibacterial activity may be indicative of the presence of some metabolic toxins or broad spectrum antibiotic compounds (ref20). Many medicinal plants as Syzgium cumini showed good activity against many microbes (Kloucek P, etal) as reported by Rajakaruna etal also. In previous findings flower, roots, and stem of some medicinal plants showed a range of activity against several bacteria and protozoa.( Diallo D etal).

In this study methanolic extract of diacure a polyherbal formulation of 11 medicinal plants showed antibacterial activity against Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus faecalis, Micrococcus luteus, Escherichia coli and Candida albicans, of concentrations 80, 90 and 100µl/disc. Of which methanolic extract of 100µl/disc concentration showed greatest activity against Pseudomonas aeruginosa, Escherichia coli as compared with other organisms tested. Then 80µl/disc concentration showed greatest activity against Klebsiella pneumoniae, Micrococcus luteus, Escherichia coli and Candida albicans, as compared with other organisms tested. Finally 90µl/disc concentration showed greatest activity against Klebsiella pneumoniae, Escherichia coli and Candida albicans. Overall the methanolic extract showed greatest activity against Klebsiella pneumoniae, Escherichia coli and Candida albicans. This is in agreement with the previous reporters of same microbes with different medicinal plants(Diallo D etal).

**Figure 1 showing the antimicrobial activity of diacure a polyherbal formulation**

**Fig 2a : Hple of standard samples**

**Peak identities:** GA, gallic acid; Cat, catechin; CA, caffeic acid; ChA, chlorogenic acid; Rut, rutin; Pzn, phloridzin; Qt, quercetin; Nar, naringenin.

The HPLC ‘fingerprint’ (Fig. 2b) of the methanolic extract of diacure show major peaks at the retention times (min.) of 2.61 at a wavelength of 280 nm. Out of the the extracts, diacure
showed maximum amount of naringenin (400 µg/g), tannic acid (319.33 µg/g) followed by gallic acid (20.76 µg/g) and ferulic acid (37.55 µg/g) (Fig. 2b). Diacure also revealed three types of Phenolic acids in which tannic acid (285.90 µg/g) was present in maximal amount whereas gallic acid and caffeic acid were in trace (Fig. 2b) HPLC analysis of the samples revealed wide-variability in their Phenolic acid content (Fig. 2a). The results of the antibacterial activity of the various crude extracts were in agreement with the uses of the extract of Diacure in traditional medicine. The rhizome and aerial parts of the plants appeared to be a potential source of broad spectrum antibiotics.

**Conclusion**

In conclusion, this study provides new scientific information about diacure, based on its antimicrobial potential and chemical profiling that has never been reported. The antibacterial activity of *diacure* may be attributed to the various phytochemical constituents present in the crude extract. The purified components may have even more potency with respect to inhibition of microbes. Further work on the types of phytoconstituents and purification of individual groups of bioactive components can reveal the exact potential of the plant to inhibit several pathogenic microbes and encourage in developing a novel broad spectrum antimicrobial herbal formulation in future.

**References**

16. Khan, N.S., Ahmed, A., Hadi, S.N. Antioxidant, pro-oxidant characterization, and therefore this study also established HPLC form2b: HPLC of methanolic extract of diacure a polyherbal formulation

According to Bauer and Tittel and Springfield et al., HPLC fingerprinting is the best way for chemical characterization, and therefore this study also established HPLC fingerprint for the active polyherbal acids that can act as antioxidant, antifungal, antibacterial and anti-inflammatory. diverse pharmacological activities have been accredited to polyherbal acids for instance, gallic acid has anti-inflammatory 23 , antibacterial 24 , caffeic acid with anti inflammatory 13 , antibacterial, antifungal 24 ; ferulic acid with anti-inflammatory 11 , antifungal 25 ; cinnamic acid with antifungal 26 , anthelmintic ,natural protection against infections by pathogenic microorganisms 27 , salicylic acid with antipyretic and antiinflammatory 28 , externally used as antiseptic, antifungal and for various skin conditions 29 .  

Naringenin are considered as one of the main groups of compounds responsible for the sedative activity of *Valeriana*. For the quantitative determination of valepotriates a direct spectrophotometric scanning on TLC plates was compared with HPLC. In *Tectonagrandis*, centrifugal partition chromatography was used to isolate the active compound such as deoxypulachol and tectoquinine that indicated fungal cell wall stress. The actively ingredients are called valepotriates, research has confirmed that these have a calming effect on agitated people, but are also a stimulant in cause of fatigue. The leaves of the swietenia macrophylla plant possess anti diabetic, anti-inflammatory, antipyretic, analgesic, antispasmodic, antimicrobial, cathartia and expectorant activities.  

**Conclusion**

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**References**