ANTIFUNGAL PHYTOCOMPONENTS IN *n*-BUTANOL FRACTION OF LEAF EXTRACT OF *KOCHIA INDICA* WIGHT.

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ABSTRACT

This study reports antifungal activity of *n*-butanol fraction of methanolic leaf extract of *Kochia indica* Wight against *Macrophomina phaseolina* and its phyto-components profiling through GC-MS analysis. Eight concentrations of the extract, ranged from 1.562 to 200 mg mL^{-1} were prepared in liquid malt extract medium. Bioassays revealed the significant inhibitory activity of all the concentrations resulting in 63-92% reduction in biomass of *M. phaseolina*. GC-MS analysis revealed the presence of 29 compounds in the fraction. Among these pentane, 2-butoxy- (8.55%); propane, 2,2-diethoxy- (8.21%); butane, 1-(1-ethoxyethoxy) (8.19%); 5-methyl-2-hexanol (7.48%) and 1-butanol, 3-methyl-acetate (7.36%) were the most abundantly occurring compounds, which may possibly be responsible for antifungal behavior of *n*-butanol fraction of methanolic leaf extract of *K. indica* against *M. phaseolina*.

Keywords: Antifungal compounds, *Kochia indica, Macrophomina phaseolina,* natural fungicides.

INTRODUCTION

Kochia indica Wight belongs to the family Chenopodiaceae. This family includes a large number of Halophytes (Yaqoob *et al*., 2013). Studies have shown that *K. indica* extract is rich in alkaloids (Youssef, 2013), flavonoids (Chou and Talalay, 1984), and saponins and tannis (Abou-Zid, 2011). Therefore, it has many biological properties and act as antioxidant, heart tonic and anticancerous agent (Youssef, 2013). Previous investigations indicated that *K. indica* has a great medicinal potency and strong tumoricidal properties (Haroun, 2010).

Macrophomina phaseolina is a pathogenic fungus that attacks more than 500 plant species, which include soybean, sorghum, corn, cotton, sunflower, sesame, common bean and others (Sánchez *et al*., 2017). Diseases caused by *M. phaseolina* are typically associated with high temperature and drought (Fang *et al*., 2011). Fungicides such as carbendazim (PCNB), quintozene, captan [N (trichloromethylthio) cyclohex-4-ene-1,2- dicarboximide] and tetramethylthiuram disulfide can effectively control the charcoal rot fungus *M. phaseolina* (Dubey *et al*., 2012). Similarly, Zveibil *et al*. (2012) reported that metam sodium and methyl bromide can be used for management of *M. phaseolina*. Maneb fungicide also inhibited 100% *in vitro* growth of *M. phaseolina* (Vasebi *et al*., 2013). However, chemical fungicides are great threat for public health and have adverse effects on environment that may lead to an immediate and long term effects (Sahli *et al*., 2010). Therefore, some alternative environmental friendly measures are needed to combat the menace. Natural plant-derived products displayed antifungal activity without being toxic and could be integrated into pest management programs as they are safe (Askarne *et al*., 2012). Antifungal potential of plant-derived compounds have been evidenced in various previous studies (Maya and Thippanna, 2015; Ali *et al.*, 2017). According to Nefzi *et al.* (2016), *Withinia somnifera* extracts significantly reduced *Fusarium oxysporum* mycelial growth. Similarly, leaf extracts of *Azadirachta indica* and *Datura metel* displayed good antifungal activity against *M. phaseolina* (Lakshmeesha *et al*., 2013). Likewise, aqueous leaf extract of *Tamarindus indica* showed excellent inhibitory activity against *Alternaria citri*, *Gibberella avenaceum* and *Fusarium incarnatum* (Satpute and Vanmare, 2017). Various recent studies have shown that extracts of *Cirsium arvense*, *Sisymbrium irio* and *Azadaricta indica* can effectively control growth of *M. phaseolina* (Banaras *et al.*, 2017; Javaid *et al*., 2017; Munir *et al.,* 2018). However, studies regarding the antifungal activity of *K. indica* extracts against *M. phaseolina* are lacking. The present study aimed to investigate and identify the possible chemical constituents responsible for antifungal activity in *n*-butanol fraction of *K. indica* leaf extract against *M. phaseolina*.

MATERIALS AND METHODS

Methanolic extract was prepared by soaking dry and powdered leaves (1 kg) in methanol (2 × 3L) at room temperature for two weeks. Following filtration, the methanolic extract was evaporated in a rotary evaporator to acquire 196 g crude extract of leaf (Javaid *et al*., 2012). The crude methanolic leaf extract (196 g) was mixed in 200 mL of distilled water, and the resultant was partitioned with *n*-hexane (6 × 400 mL) by separating funnel to

afford *n*-hexane fraction and aqueous part. Then the remaining aqueous phase was successively partitioned with chloroform (400 mL), ethyl acetate (400 mL) and *n*-butanol (400 mL) (Akhtar and Javaid, 2018). In order to evaluate *in vitro* activity of *n*-butanol fraction acquired from methanolic leaf extract against *M. phaseolina*, one milliliter of dimethyl sulphoxide (DMSO) was used to suspend 1.2 g of *n*-butanol fraction. The suspended material was carefully mixed with five milliliter of malt extract (ME) broth, in order to prepare 6 mL of stock solution at 200 mg mL^{-1} concentration. Broth medium was divided into two equal portions; one half was serially double diluted for preparing lower concentrations up to 1.562 mg mL^{-1} and the other half was utilized for experimentation. The said experiment was carried out in autoclaved glass test tubes. Each test tube contained one millimeter of ME broth. Inoculum of *M. phaseolina* was made in autoclaved distilled water, 50 µL of the inoculum was appended to each test tube and incubated at 28°C. Each treatment was replicated thrice. Fungal biomass was filtered and weighed after seven days of incubation (Shafique *et al*., 2016). All the data were analyzed by ANOVA followed by LSD test (P = 0.05) using computer software Statistix 8.1.

RESULTS AND DISCUSSION

n-Butanol fraction showed considerably high antifungal activity against *M. phaeolina* (Fig. 1 and 2). All the concentrations significantly reduced the biomass of the fungus by 63-92% over corresponding control treatments. Inhibitory effect became more pronounced as the concentration of the extract was increased. In previous studies, extracts of other species of *Chenopodium* namely *C. murale* and *C. ambrosioides* decreased biomass of *M. phaseolina* by 62-90% and 50-84%, respectively (Javaid and Amin, 2012). Earlier, mixture of sterols, mainly sitosterol, *n*-alkanes and free alcohols were isolated from the aerial parts of *Kochia*. Due to the presence of these hydrocarbons and sterols, *Kochia* exhibited antibacterial activity (Abdel-Hamid *et al*., 2017). The GC-MS analysis of *K. indica* extract done by Haroun (2010) detected an active phenolic compound, 1-phenyl 2,3,4,5,6 hexachloro 2,3,4,5 tetra hydroxy heptane that may be responsible for antifungal activity.

Table 1. Compounds identified from *n*-butanol fraction of methanolic leaf extract of *Kochia indica* through GC-MS analysis.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	5-Methyl-2-hexanol	$C_7H_{16}O$	116	5.124	7.48
2	1-Hexanol	$C_6H_{14}O$	102	5.242	4.78
3	1-Butanol, 3-methyl-acetate	$C_7H_{14}O_2$	130	5.418	7.36
4	Pentane, 1-propoxy-	$C_8H_{18}O$	130	5.562	4.40
5	3-Hexanol	$C_6H_{14}O$	102	5.882	5.57
6	Propane, 2,2-diethoxy-	$C_7H_{16}O_2$	132	6.050	8.21
7	Propane,1,1'-[ethylidenebis(oxy)]bis-	$C_8H_{18}O_2$	146	6.255	6.97
8	Heptane, 2-iodo	$C_7H_{15}I$	226	6.384	3.33
9	Butane, 1-(1-ethoxyethoxy)	$C_8H_{18}O_2$	146	6.452	8.19
10	Pentane, 2-butoxy-	$C_9H_{20}O$	144	6.731	8.55
11	Cyclohexane, propyl-	C_9H_{18}	126	6.861	1.98
12	1-Nonene	C_9H_{18}	126	6.931	2.50
13	2-Methylbutane-1,4-diol, 3-(1-ethoxyethoxy)-	$C_9H_{20}O_4$	192	7.023	3.49
14	Pentane, 1-butoxy	$C_9H_{20}O$	144	7.115	3.56
15	Propane,1,1-diethoxy-2-methyl-	$C_8H_{18}O_2$	146	7.249	3.92
16	1-Pentanol, 2-methyl-, acetate	$C_8H_{16}O_2$	144	7.358	1.50
17	Silane, triethyl-	$C_6H_{16}Si$	116	7.407	1.30
18	2-Methyl nonane	$C_{10}H_{22}$	142	7.742	1.37
19	2,3-Dibutyloxirane	$C_{10}H_{20}O_2$	156	7.793	1.91
20	3-Ethyl-5-methyl heptane	$C_{10}H_{22}$	142	7.887	1.55
21	1-Butoxypentane	$C_9H_{20}O$	144	8.140	2.81
22	Acetic acid, hexyl ester	$C_8H_{16}O_2$	144	8.184	1.81
23	Propane, 1,1-diethoxy-	$C_7H_{16}O_2$	132	8.449	1.72
24	n-Decane	$C_{10}H_{22}$	142	8.571	3.34
25	1-(1-Ethoxyethoxy)octane	$C_{12}H_{26}O_2$	202	8.814	0.79
26	6-Hydroxyhexahydrocyclopenta[b]furan-2-one	$C_7H_{10}O_3$	142	8.893	0.41
27	Cyclohexene, 1-butyl-	$C_{10}H_{18}$	138	9.440	0.41
28	Dioctyl phathalate	$C_{24}H_{38}O_4$	390	26.006	0.31
29	β-Sitosterol	$C_{29}H_{50}O$	414	31.657	0.47

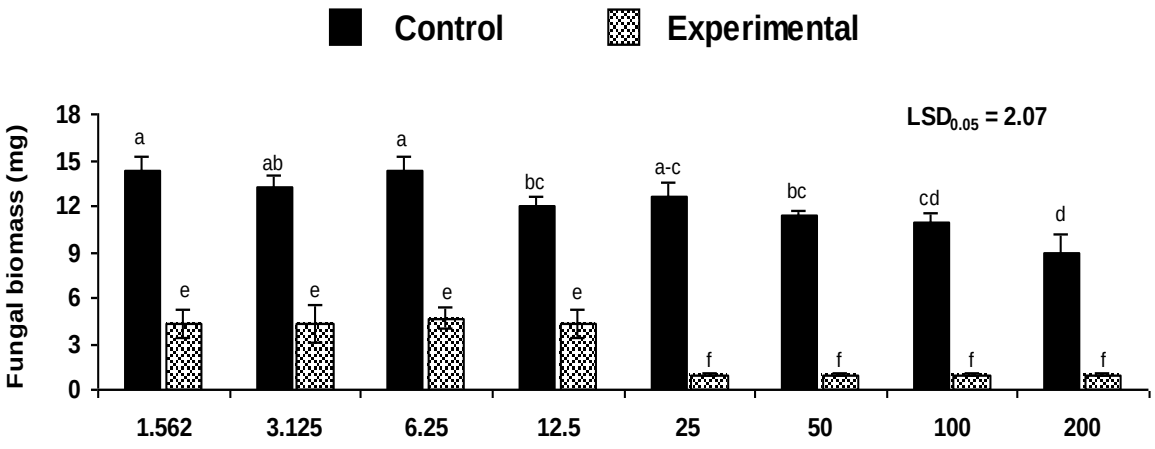


Fig. 1. Effect of different concentrations of *n*-butanol fraction of methanolic leaf extract of *Kochia indica* on biomass of *Macrophomina phaseolina*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

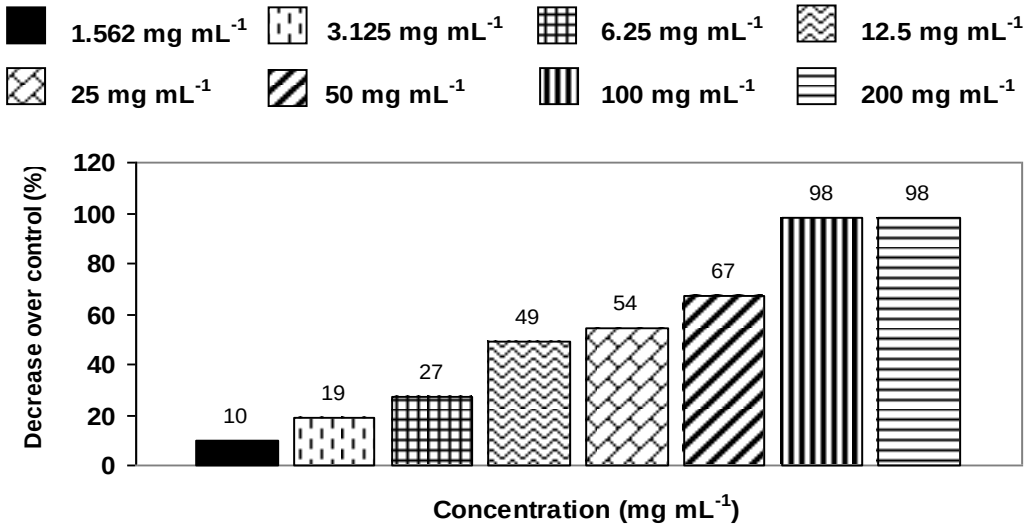


Fig. 2. Percentage decrease in biomass of *Macrophomina phaseolina* due to different concentrations of *n*-butanol fraction of methanol leaf extract of *Kochia indica* over control.

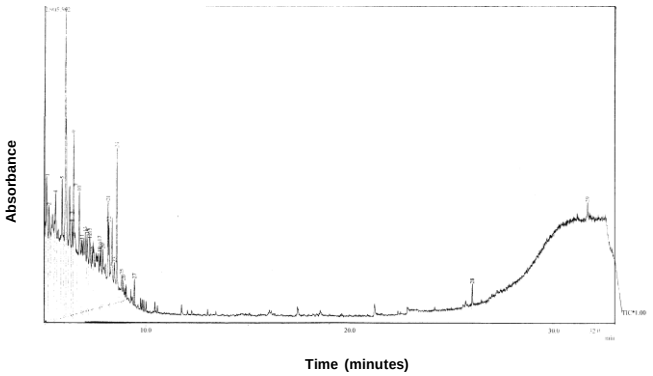
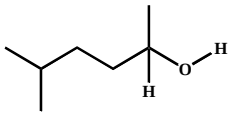
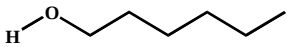


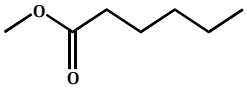
Fig. 3. GC-MS chromatograms of *n*-butanol fraction of methanolic leaf extract of *Kochia indica.*



1. 5-Methyl-2-Hexanol

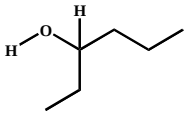


2. 1-Hexanol

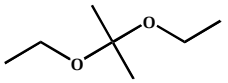


3. 1-Butanol, 3-methyl-acetate

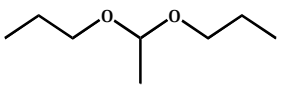
4. Pentane, 1-propoxy-



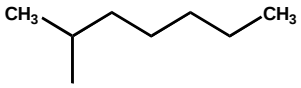
5. 3-Hexanol



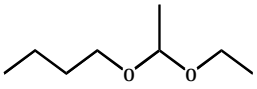
6. Propane, 2,2-diethoxy-



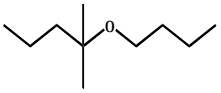
7. Propane,1,1'-[ethylidenebis(oxy)]bis-



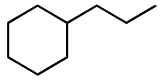
8. Heptane, 2-iodo



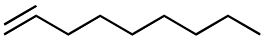
9. Butane, 1-(1-ethoxyethoxy)



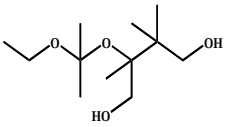
10. Pentane, 2-butoxy



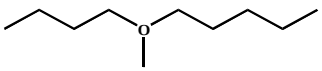
11. Cyclohexane, propyl-



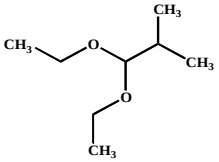
12. 1-Nonene



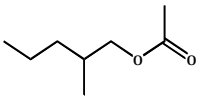
13. 2-Methylbutane-1,4-diol, 3-(1-ethoxyethoxy)-



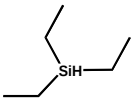
14. 1-butoxy pentane



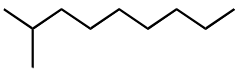
15. 1,1-Diethoxy-2-methyl propane



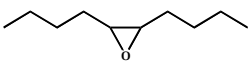
16. 1-Pentanol, 2-methyl-, acetate



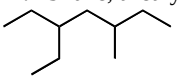
17. Silane, triethyl



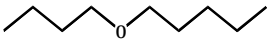
18. 2-Methyl nonane



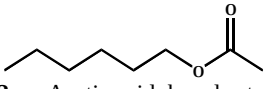
19. 2,3-Dibutyloxirane



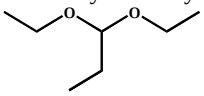
20. 3-Ethyl-5-methyl heptane



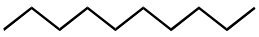
21. 1-Butoxypentane



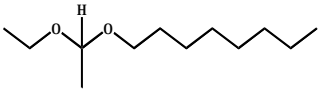
22. Acetic acid, hexyl ester



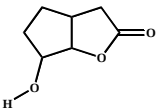
23. Propane, 1,1-diethoxy-



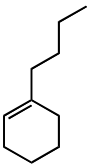
24. *n*-Decane



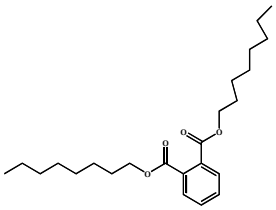
25. 1-(1-Ethoxyethoxy)octane



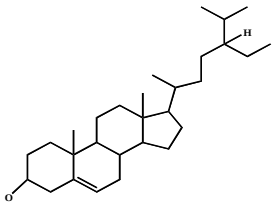
26. 6-Hydroxyhexahydrocyclopenta[b]furan-2-one



27. Cyclohexene, 1-butyl-



28. Dioctyl phathalate



29. β-sitosterol

Fig. 4. Structures of compounds identified in *n*-butanol sub-fraction of methanolic leaf extract of *Kochia indica* through GC-MS.

GC-MS chromatogram of *n*-butanolic fraction indicated the presence of twenty nine phytochemical constituents (Fig. 3). Details of various identified compounds are given in Table 1. Structures of these compounds are presented in Fig. 4. Pentane, 2-butoxy- (8.55%); propane, 2,2-diethoxy- (8.21%); butane, 1-(1-ethoxyethoxy) (8.19%); 5-methyl-2-hexanol (7.48%), and 1-butanol, 3-methyl-acetate (7.36%) were the most abundantly occurring compounds. The compounds namely propane, 1, 1'-[ethylidenebis(oxy)]bis- (6.97%); 3-hexanol (5.57%); 1-hexanol (4.78%) and pentane, 1-propoxy- (4.40%) were moderately abundant. Compounds present in lower concentration were propane, 1,1-diethoxy-2-methyl- (3.92%); pentane, 1-butoxy (3.56%); 2-methylbutane-1,4-diol, 3-(1-ethoxyethoxy)- (3.49%); heptane, 2-iodo (3.33%); 1-butoxypentane (2.81%); 1-nonene (2.50%); cyclohexane, propyl- (1.98%); 2,3-dibutyloxirane (1.91%); 3-ethyl-5-methyl heptanes (1.55%); acetic acid, hexyl ester(1.81%); propane, 1,1-diethoxy- (1.72%); 1-pentanol, 2-methyl-, acetate (1.50%); 2-methyl nonane (1.37%) and silane, triethyl- (1.30%). The least occurring compounds were 1-(1-ethoxyethoxy) octane (0.79%); β-sitosterol (0.47%); 6-hydroxyhexahydrocyclopenta[b]furan-2-one (0.41%); cyclohexene, 1-butyl- (0.41%) and dioctyl phathalate (0.31%). Dioctyl phthalate was reported as a major constituent in *L. nudicaulis* which is responsible for antimicrobial activity (Zellagui *et al*., 2012). Phthalates are known to possess antimicrobial and other pharmacological activities. The essential oil of *Leea indica* showed phthalic acid esters as major constituents, which had potent antifungal activity (Srinivasan *et al*., 2009**).**

The present study concludes that *M. phaseolina* can be managed by the *n*-butanolic fraction of methanolic leaf extract of *K. indica*.

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