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Phytochemical study of Alysicarpusmonilifer

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ABSTRACT

Plant species and their active constituents play an important role in the prevention of a variety of ailments. The genus *Alysicarpus* comprises of about 30 species, out of which, eight species are found in Pakistan. Only a few species of the genus *Alysicarpus* have been investigated for phytochemical and biological activities. A variety of potentially bioactive molecules such as alkaloids, flavonoids, phenolics, terpenoids and steroids have been isolated from various species of genus *Alysicarpus.* It has been reported that these species possess analgesic, anti-inflammatory, antimicrobial, antiplasmodial, larvicidal, mosquitocidal, antioxidant, hepatoprotective, antiproliferative and antifertility activity. This article aims to highlight the phytochemical aspects of *Alysicarpus* species and illustrates the potential of the genus as a source of therapeutic agents.

Keyword: Alysicarpus,Phytochemical studyaspects

I. INTRODUCTION

Natural products coming from various sources, such as terrestrial plants, microorganisms, marine organisms, vertebrates and invertebrates, have importance as they provide an amazing source of new drugs as well as new drug leads and chemical entities for further drug development

To evaluate the phytochemical activity of different extract of aerial parts of Alysicarpus monilifer belonging to the family Fabacea. The aerial parts were collected and extract prepared from petroleum ether, ethyl acetate and methanol by hot continuous percolation method in a Soxhlet apparatus for 24 hrs. The preliminary phytochemical investigation shows presence of alkaloids, proteins & free amino acids, glycosides, phytosterols, saponins,carbohydrates& free reducing sugars, tannins & phenolic compounds and flavonoids. Each active compound shows different activities against different types of diseases like cancer, liver disorders, diabetes, atherosclerosis and inflammatory diseases etc. According to their characteristics, they can be involved in the medicinal plant category.

Phytochemical studies on genus Alysicarpusmonilifer

The seven species of genus Alysicarpus namely A. bupleurifolius, A. monilifer, A. longifolius, A. ovalifolius, A. procumbens, A. rugosus and A. vaginalis have been phytochemically investigated.

Phytochemical screening

The previous phytochemical studies, reported so far, from above mentioned species of Alysicarpus showed the prescence of different classes of secondary metabolites such as alkaloids, cardiac glycosides, flavonoids, tannins, saponins, terpenoids and steroids. The details of phytochemical screening of Alysicarpus species are give Isolation of phytochemicals.

The phytochemicals isolated from various species of Alysicarpus belong to different classes of secondary metabolites such as alkaloids, flavonoids, phenolics, terpenoids and steroids as described below.

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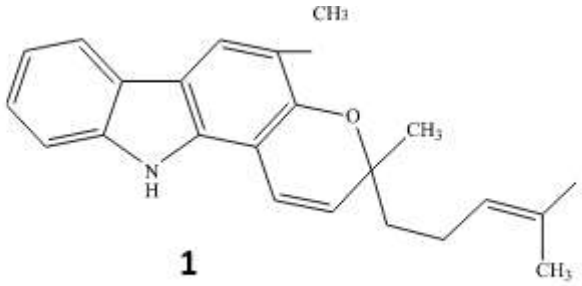

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The phytochemicals isolated from various species of Alysicarpus belong to different classes of secondary metabolites such as alkaloids, flavonoids, phenolics, terpenoids and steroids as described below.

Alkaloids : The evalution of stem bark of A. ovalifolius has led to theisolation of three carbazole alkaloids namely, mohanimbine(1), koenimbine (2) and koenidine (3). Mohanimbine displayed larvicidal activity and significant antimicrobial activities against gram positive Staphylococcus aureus andCandida albicans with zone of inhibition ranging from 10.4 to 13.8 mm in diameter at a concentration of 100 µg/ml.

Koenidine showed promising activity against Plasmodium falciparum with IC50 54.19 ng/ml.

Table No 01: Percentage yield of the whole plant of A.monilifer

Plant name	Parts used	Method of extraction	Solvent system	Percentage yield (%w/w)
A.monilifer	Whole plant	Continuous hot percolation	Petroleum ether Ethyl acetate methanol	5.38 7.16 14.52

Table No 02 : Phytochemical compound identified in petroleum ether extract of A.monilifer by gas Chromatography- mass spectrometry (GC-MS) analysis

S.NO	Compound name	Probability	Molecular formula	MOL.wt	Area %	Run Time
1	1-Methoxy-3-pentene	47.43	$C_6H_{12}O$	100	0.14	3.32
2	5-Nonanone	8.89	$C_9H_{18}O$	142	0.14	3.32
3	3-Methylbut-3-en-2-ol	8.54	$C_6H_{12}O$	100	0.14	3.32
4	2-Propenoic acid	7.55	$C_4H_{12}O2$	86	0.14	3.32
5	6-chlorohex-2en-1-ol	5.63	$C_6H_{11}C_{10}$	134	0.14	3.32
6	1,3-Propanediamine, N,N-dimethyl-	5.19	$C_5H_{14}N_2$	102	0.14	3.32
7	2,6-Dideutero-pyridine	49.50	$C_5H_3D_2N$	79	97.22	3.80
8	IS-(-)-N-(Cyclohex-2-en-1-yl)hydroxylamine	9.88	$C_6H_{11}NO$	113	97.22	3.80
9	BENZENE-1,3,5-D3	9.88	$C_6H_3D_3$	78	97.22	3.80
10	(Z)-2-cyano-2-butene	6.18	C_5H_7N	81	97.22	3.80
11	4-dl-3-cyclohexen-1-ol	4.24	C_6H_9DO	98	97.22	3.80
12	2-vinylpent-3-en-1-ol	2.55	$C_7H_{12}O$	112	97.22	3.80
13	Fenchone	2.45	$C_{10}H_{16}O$	152	97.22	3.80
14	1-phenylethyl N,N-diisopropylcarbamate	6.26	$C_{15}H_{23}NO_2$	249	0.02	5.74

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15	3-Pentanone, 2-methyl-4-phenyl	4.16	$C_{12}H_{16}O$	176	0.02	5.74
16	Benzene,(1,2,2-trimethyl-3-butenyl)	3.36	$C_{13}H_{18}$	174	0.02	5.74
17	Phenethylsulfanyl methylene -1,3-dioxane-4,6-dione	2.83	$C_{15}H_{16}O_4S$	292	0.02	5.74
18	N-BZ-2-aminocinnamate	2.72	$C_{17}H_{17}NO_2$	267	0.02	5.74
19	1-(a- Methylbenyl)-2-(methoxymethyl)-aziridine	2.62	$C_{12}H_{17}NO$	191	0.02	5.74
20	4-Cyclopropylcarbonyl Oxytridecane	11.69	$C_{17}H_{32}O_2$	268	0.13	6.59
21	2,2,3,3-Tetraethyloxirane	9.88	$C_{10}H_{20}O_2$	156	0.13	6.59
22	Butane, 1,2-dichloro-2-methyl	7.17	$C_5H_{10}CL_2$	140	0.13	6.59
23	Dodecane,1-chloro	5.63	$C_{12}H_{25}CL$	204	0.13	6.59
24	Dimethyldiphenyltethylidylpyrrolidine	5.20	$C_{20}H_{23}N$	277	0.13	6.59
25	1,3,5-Triazine-2,4(1h,3h)-dione,6-(methylamino)	3.98	$C_4H_6N_4O_2$	142	0.13	6.59
26	Beneneacetic acid,-4-tetradecyl ester	3.98	$C_{22}H_{36}O_2$	332	0.13	6.59
27	Octadecane,1-chloro-Hexadecane	3.52	$C_{18}H_{37}CL$	288	0.13	6.59
28	Acetic acid	3.52	$C_{16}H_{34}$	226	0.03	16.95
29	1=(1'-methylalleneyl)-2-ethenylcyclohexane	42.27	$C_{16}H_{26}O_2$	250	0.05	18.78
30	A'-ylangene	6.57	$C_{12}H_{18}$	162	0.05	18.78
31	A'-guaiene	5.80	$C_{15}H_{24}$	204	0.05	18.78
32	2,7-dimethyl1-3,6-dimethylene-1,7-octadiene	4.56	$C_{15}H_{24}$	204	0.05	18.78
33	Undecane	3.88	$C_{12}H_{18}O$	162	0.05	18.78
34	7,7-dichlorobicyclo hep-2-en-6-one	3.73	$C_{15}H_{24}O$	220	0.05	18.78
35	7,7-dichlorobicyclo hep-2-en-6-one	3.29	$C_{15}H_{24}0$	220	0.05	18.78
36	10,12-Tricosadiynoic acidmethyl ester	2.52	$C_{14}H_{22}O_3$	360	0.05	18.78
37	2-but-3enyl-1-4dioxaspiro acetaldehyde	47.14	$C_{11}H_{19}NO_3$	238	0.02	20.01
38	N-Carbethoxy-7-azabicyclo-9-ol	10.22	$C_{13}H_{22}N_2O_5$	213	0.02	20.01
39	1-(4-hydroxybutyl)-5-(ethoxyethoxymethyl)uracil	9.03	$C_{15}H_{13}O_3S$	286	0.02	20.01
40	S-Methyl-3-(2-naphthyl) Thiacyclobutenium	6.55	$C_{15}H_{13}F_3O_3S$	362	0.02	20.01

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Table No 03 : Effect of pet.ether extract of A.monilifer on DPPH assay

S.No	CONCENTRATION (µg/ml	% OF ACTIVITY(±SEM)	
		SAMPLE (PET.ETHER EXTRACT)	STANDARD (RUTIN)
1	250	31.56 ± 0.060	22.08 ± 0.054
2	500	31.56 ± 0.025	52.21 ± 0.022
3	1000	45.55 ± 0.041	69.83 ± 0.014
	IC_{50}=1120 µg/ml		IC_{50}=480 µg/ml

Table no 04 : effect of ethyl acete extract of A.monilifer on iron-chelating

S.NO	CON (µg/ml)	b % of activity (± SEM)	
		SAMPLE (PET.ETHER EXTRACT)	STANDER (EDTA)
1	250	41.65 ± 0.074	65.87 ± 0.018
2	500	50.53 ± 0.032	83.8 ± 0.012
3	1000	61.54 ± 0.063	97.90 ± 0.019
	IC_{50}=490 µg/ml		Ic_{50} =65 µg/ml

All values are express as mean ± SEM for three determination

Table no 05 : Effect of pet ether of A.monilifer on hydroxyl radical scavenging

Activity

S.NO	CON (µg/ml)	% of activity (±SEM)	
		SAMLPE	STANDARD
1	125	19.47 ± 0.031	26.87 ± 0.076
2	250	29.68 ± 0.024	30.30 ± 0.054
3	500	35.96 ± 0.033	60.64 ± 0.022
	IC_{50}=1070 µg/ml		IC_{50}=410 µg/ml

All values are expresses as mean ± SEM for three dermination

Table no 06 : effect of pet.ether extract of A.moniliferon nitric oxide scavenging

S.NO	CON (µg/ml)	% of activity (±SEM)	
		SAMLE	STANDARD
1	125	23.08 ± 0.034	26.87 ± 0.076
2	250	32.84 ± 0.028	30.30 ± 0.054
3	500	40.22 ± 0.046	60.64 ± 0.022
	IC_{50}= 970 µg/ml		IC_{50}= 410 µg/ml

All values are expresses as mean ± SEM FOR three determination

Table no 07: Effect of pet ether extract of A.monilifer on total antioxidant activity

S.NO	CON (µg/ml)	% of activity (±SEM)	
		SAMLPE	STANDARD
1	125	13.30± 0.022	26.87 ± 0.076
2	250	26.98 ± 0.030	30.30 ± 0.054
3	500	42.84 ± 0.024	60.64 ± 0.022
	IC50= 1025µg/ml		IC50= 410 µg/ ml

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*All values are expressed as mean ± SEM for three determinations

Table no 08 : effect of pet. Ether extract of A.monilifer on FRAP assay

S.NO	CON (µg/ml)	% of activity (±SEM)	
		SAMLPE(pet ether extract)	STANDARD (ascorbate)
1.	125	20.94 ± 0.033	72.04 ± 0.014
2.	250	24.68 ±0.026	82.05 ± 0.034
3.	500	35.46 ± 0.018	86.04 ± 0.026
	IC50=1110 µg/ml	IC50=50µg/ml	

All values are expressed as mean ± SEM for three determionation

Table no 09 : The total phenolic content of various extracts of A.monilifer

S.NO	EXTRACTS	Total phenol content (mg/g of catechol) (± SEM)
1.	Petroleum ether extract	2.218 ± 0.022
2.	Ethyl acetate extract	3.54 ± 0.042
3.	Methanolic extract	4.98 ± 0.033

All values are expressed as mean ± SEM for three determinations

Table no:10 : The total flavonoids content of various extracts of A.monilifer

S.NO	Extracts	Total flavonoids content (mg/g)(±SEM)
1.	Petroleum ether extract	0.046 ± 0.021
2.	Ethyl acetate extract	1.114 ± 0.026
3.	Methanolic extract	3.751 ± 0.038

*All values are expressed as mean ± SEM for three determinations

Table No 11: Effect Of various extracts of A.monilifer on fasting blood glucose level in diabetic rats

Fasting blood glucose level (mg/dl)

Groups	Day 1	Day 7	Day 15	Day 30	Day 45	Day 60	Day 70
Group1	**78.92 ± 25.05**	**81.29 ± 22.10**	**82.88 ± 22.10**	**82.88 ± 25.30**	**82.20 ± 24.63**	**82.12 ± 25.55**	**81.92 ± 25.33**
Group 2	**241.06 ± 25.26**	**313.68 ± 34.48**	**327.19 ± 40.95**	**316 ± 37.88**	**308.40 ± 47.26**	**302.12 ± 47.26**	**289.10 ± 23.20**
Group 3.	**243.52 ± 25.44**	**296.52 ± 35.40**	**216.14 ± 30.90**	**169.14 ± 40.67**	**151.54± 27.14**	**125.36 ± 19.10**	**108.46 ± 27.16**
Group 4.	**237.62 ± 23.10**	**302.53 ± 23.30**	**270.79 ± 28.50**	**255.14 ± 27.22**	**223.18 ± 29.50**	**202.66 ± 17.64**	**195.53 ± 23.32**

values are given as means S.D from six rats in each group.
p≤ 0.05, p≥ 0.01 when compared to STZ control group

Table no 12 effect of various extracts of A.monilifer on vitamin C in the plasma and tissues of control and diabetic rats.

Group	Plasma (mg/dl)	Tissues (mg/g protein)	
		Liver	Kidney
Group 1	**1.98 ± 0.19**	**0.92 ± 0.08**	**0.83 ± 0.05**
Group 2.	**0.88 ± 0.06**	**0.44 ± 0.04**	**0.68 ± 0.04**
Group 3	**1.85 ± 0.08**	**0.86 ±0.03**	**0.73 ± 0.03**
Group 4	**2.26 ± 0.24**	**1.25 ± 0.05**	**1.18 ± 0.05**
Group 5	**1.96 ± 0.22**	**1.02 ± 0.04**	**0.92 ± 0.05**
Group 6	**1.70 ± 0.05**	**0.76 ± 0.05**	**0.72 ± 0.06**

Values are given as means S.D from six rats in each group
P<0.05, **P<0.01when compared to STZ control group

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Table no 13 : effect of various extracts of A.monilifer on (Na+)-ATPase in the erythrocytes and tissues of control and diabetic rats

Groups	BLOOD ERYTHROCYTES (U^a/mg protein)	TISSUES LIVER (U^a/mg protein)	KIDNEY (U^a/mg protein)
Group 1 (control)	0.90 ± 0.09	0.86 ± 0.08	0.57 ± 0.05
Group 2	0.38 ± 0.04	0.36± 0.03	0.29 ± 0.03
Group 3	0.77 ± 0.08	0.74 ± 0.07	0.61 ± 0.06
Group 4	0.62 ± 0.09	0.61 ± 0.06	0.41 ± 0.04
Group 5	0.66 ± 0.07	0.68 ± 0.07	0.41 ± 0.05
Group 6	0.73 ± 0.08	0.72 ± 0.08	0.51 ± 0.05

Values are given as mean ± S.D from six rats in each group. *P<0.05, **P<0.01when compared to STZ control group

The present study was evaluating the beneficial effects of whole plant of methanolic extract of A.monilifer on antioxidant status in STZ induced diabetic rats.theinstenified free radical production during STZ mediated experimental diabetic results in the elevated level of lipid peroxides and conjugated diene oxidative degradation of polyunsaturated fatty acids. These are unstable, cytotoxic and highly reactive, leading to free radical damage to proteins and DNA and finally cause various diabetes mediated complications. The degree of tissue damage persuaded by free radicals depends on the balance between free radical generation and the endogenous antioxidant defense mechanism (Davi et al., 2005). One of the most often used biomarker to investigate the oxidative damage on lipid is TBARS a major lipid peroxidation product. It can react with the free amino group of proteins, phospholipids, and nucleic acids leading to structural modification (Pandey and Rizvi, 2010). According to the provided data in table 5.36.a notable increase in TBARS level in liver, kidney and pancreas were observed in STZ-diabetic rats compared with their respective normal controls. Previous study had reported increased levels of lipid peroxidaion in STZ diabetic rats (Hussein, 2008). However, the oral administration of A.monilifer to the diabetic group of rats significantly reverted back TBARS levels to near normal values which show the anti lipidperoxidative property of A.monilifer methanolic extract in experimental diabetes. STZ diabetic rats treated with A.monilifer methanolic extract had normal plasma and tissue organs GSH levels. This effect may be because it rapidly reacting with O2. The affinity of GSH for O2 is far greater than the affinity of SOD for O2.In fact, GSH may compete with SOD for O2 -and sparing SOD for other scavenging duties . Furthermore, it was reported by (Brown and Hu, 2001) that increases bioavailability of antioxidants are expected to decrease superoxide generation by increasing hydrobiopterin (BH4) a cofactor needed to stimulate endothelial nitric oxide synthase activity. Therefore, it is likely that the antioxidant effect of A.monilifer methanolic extract contribute to the increased bioavailability of GSH. GSH is a major intracellular non protein sulphydral compound and is accepted as the most important intracellular hydrophilic antioxidant . Also, GSH acts as a co substrate for GPx and GSH Rx activity and as a cofactor for many 142 enzymes, stress resistance of many cells is associated with high intracellular levels of GSH. A decreased GSH content may predispose the cells to lower defense against condition of oxidative stress during several degenerative disease conditions including diabetes (Hussein, 2008). In the present study , the observed elevation in the activities of these antioxidant enzymes in liver and kidney of A.monilifer diabetic rats compared to the untreated ones reflects the antioxidant potential of methanolic extract of A.monilifer. Numerous studies have revealed lower antioxidant and enhanced peroxidative status in Type 2 diabetes mellitus .SOD, CAT, GPx and GST are enzymes that destroy the peroxides and play a significant role in providing antioxidant defenses to an organism are involved in the elimination of H2O2 and SO acts to dismutate superoxide radicals to H2O2 which is then acted upon by GPx. The function of all three enzymes are interconnected and lowering of their activities result in the accumulation of lipid peroxides and increased oxidative stress in diabetic rats .In the present study table (5.16 & 5.17) the activities of

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GPx, SOD and CAT in plasma and different tissue organs extracts of the STZ diabetic rats were significantly lower than their control ones. Impairment of antioxidant machinery may be described by both the damage of antioxidant enzymes caused by protein glycation and consumption by an excess demand .The compromises in enzymatic antioxidant defense system and alterations in their activities have been implicated in the mechanisms of abnormal tissue function observed in diabetes mellitus . The ubiquitous cellular enzyme (Na+)-adenosine triphosphatase (ATPase) is responsible for the maintenance of intracellular sodium and potassium concentrations . The function of this 143 enzyme is to transport three ions of sodium from the intracellular space to the extracellular environment and, in return, to allow two ions of potassium to enter the cell. The Ca2+-ATPase is the major active calcium transport protein responsible for the maintenance of normal intracellular calcium levels in a variety of cell types. Maintenance of the cation gradient by Ca2+-ATPase is of fundamental importance in the control of hydration, volume, nutrient uptake and fluidity of cells, and is also essential for the contractility and excitability properties of muscles. Membrane (Ca2++Mg2+)-ATPase is responsible for the fine-tuning of intracellular calcium and the Na+ -Ca2+ exchanger for rapid ejection of the excess calcium that has entered the cell. 5.6.4.8 Effect of various extracts of A.monilifer on histopathological examination In our study, histopathological examination of diabetic pancreas (fig. 5.28- 5.33) showed shrinkage of islet cells and growth of adipose tissue in the pancreas. Treatment with various extracts of A.monilifer and glibenclamide reduced the changes in the pancreas, which supports the biochemical analysis. Histopathological examination of diabetic liver (fig. 5.34- 5.39) showed fatty changes and inflammatory cell infiltration around the portal triad in the liver. Treatment with various extracts ofA.monilifer and glibenclamide showed marked reduction in fatty changes and inflammatory cell infiltration around the portal triad. Histopathology of diabetic kidney (fig. 5.40- 5.45) showed large area of hemorrhage, lymphocyte infiltration and fatty infiltration, which upon treatment with various extract.[106-109]

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Isolation of phytochemicals

The phytochemicals isolated from various species of *Alysicarpus* belong to different classes of secondary metabolites such as alkaloids, flavonoids, phenolics, terpenoids and steroids as described below.

Alkaloids

The evalution of stem bark of *A. ovalifolius* has led to the isolation of three carbazole alkaloids namely, mohanimbine (**1**), koenimbine (**2**) and koenidine (**3**). Mohanimbine displayed larvicidal activity and significant antimicrobical activities against gram positive *Staphylococcus aureus* and *Candida albicans* with zone of inhibition ranging from 10.4 to 13.8 mm in diameter at a concentration of 100 µg/ml. Koenidine showed promising activity against *Plasmodium falciparum* with IC_{50} 54.19 ng/ml

II. CONCLUSION

In conclusion, this review reveals that the genus Alysicarpus is endowed with many medicinal plants some of which have potential for the discovery of new drugs. On the basis of results regarding in vitro and in vivo efficacy and toxicity studies, A. vaginalis holds the most promising compounds with antiproliferative, hepatoprotective and antioxidant activity that may be due to the presence of phenolics such as tannins and flavonoids. The alkaloids of ovalifolius were reported to possess antimicrobial potential. Flavonoids, vitexin and iso- vitexin, from A. monilifer are so far the most promising compounds that could be developedas hepatoprotective agents. The other Alysicarpus species and isolated compounds require further studies to validate or ascertain their medicinal potentials.

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In the present study an attempt has been made for morpho-anatomicalas well as physicochemical evaluation of this plant for contribution in the phytochemical quality control and knowledge of Fabaceae family.

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