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Gas Chromatography-Mass Spectrometry Analysis Of Methanolic Seed And Root Extracts Of Rorippa indica L.

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Abstract: *Rorippa indica* L. is one of the medicinal plant belonging to the family Brassicaceae, commonly known as water cress. Methanolic seed and root extracts of *Rorippa indica* was analyzed by using GC-MS to identify various phytochemical compounds present in it. Twenty eight and twenty nine phytochemical compounds were identified from methanolic seed and root extract respectively.

Key words: GC-MS analysis, Bioactive Components, Rorippa indica, Methanolic Extract, Jasmonic Acid.

Introduction

Rorippa indica L. is a wild crucifer found in all continents except Antartica, mainly with dense population throughout India. It is widely distributed in the plains of Coimbatore, North Arcot and Tiruchirappalli Districts of Tamil Nadu⁽¹⁾. It is aphid tolerant and contains high percentage of oleic acid (26.65 %), linoleic acid (26.94 %) and a moderate percentage of crucic acid (24.40 %)⁽²⁾. Nature has been a source of medicine for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the usage in traditional medicine. The plant-based, traditional medicine systems continues to play an essential role in health care, with about 80 % of the world's inhabitants relying mainly on traditional medicines for their primary health care $^{(3,4)}$.

Plants contain constituents such as flavonoids, saponins, tannins, terpenoids, phenolics etc, which have been assessed for their anti-oxidant, anti-mutagenic, anti carcinogenic and other biological effects⁽⁵⁾. The curative properties of plants can be assessed by their response to attacks from

insect predators and disease causing organisms. This is achieved by the accumulation of phytochemicals at the sites of infusion of plants several of which are insecticidal, anti-bacterial and antifungal⁽⁶⁾. Elicitors have been shown to be effective strategy to achieve and increase production of secondary compounds⁽⁷⁾. Elicitors have received wide acceptance because of its ability to improve productivity of the plant cell and organ culture^(8, 9). In addition to the optimization of culture conditions (e.g, medium salt bases, sucrose concentration and pH), strain improvement and the addition of biosynthetic precursors, the treatment of plant cell and organ cultures with elicitors has been shown to be an effective strategy to increase production of secondary metabolites ⁽⁷⁾. The increased production, through elicitation of the secondary metabolites from plant cell cultures has open up a new area of research which could have important economical benefits for industry⁽¹⁰⁾. Jasmonic acid has an important role in plants as inducer for insect and disease resistance. Jasmonic acid induced pest resistance in wheat plants and acted as a resistance mechanism for wheat against insect herbivores (11). Hence jasmonic acid was selected as an elicitor for in vitro studies.

Materials and Methods

Extraction of plant samples

Seeds and roots of Rorippa indica were collected from the hardened elicitor treated (Jasmonic Acid) regenerated plant. Department of Plant Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu. Seeds and roots were cleaned, shade dried and pulverized to powder by a mechanical grinder. Preliminary screening work has done for identification of phytochemicals by using different solvents such as petroleum ether, chloroform, ethanol, aquous and methonal (Datas not been presented). Among all the solvents tested methonolic extracts proved as best for identification of phytochemical compounds. Hence in the present study methonolic solvent was used for further studies. Required quantity of powder was weighed and transferred to Soxhlet extraction process and treated with methanol until the powder is fully immersed. The extract was collected and evaporated to dryness by using vacuum distillation unit. The final residue thus obtained was then analyzed by GC-MS analysis. The spectra of the compounds were matched with NIST and Wiley library. Their structures were defined by the % similarity values.

GC-MS analysis

GC-MS analysis of these extracts were performed using a Thermo GC- Trace ultra version 5.0 and Thermo MS DSQ 11, Colum TR 5- MS-Capillary non polar column (30 Mts, ID : 0.25 mm, FILM : 0.25μ m). For GC- MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas was used as the carrier gas at constant flow rate (He, flow: 1 ML/Min), injector temperature of 100 – 250 °C, rate: 8/Min, holding time: 10 min at 250 °C. Total GC - MS running time was 33.43 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a turbo mass.

Results and Discussion

The components present in the methanolic extracts of seeds and roots of *Rorippa indica* were identified by GC-MS analysis (Figure 1and 2). The active principles with their Retention Time (RT),

Molecular formula, Molecular Weight (MW) and Concentration (%) in the seeds and roots of R. indica presented and tabulated. Twenty are eight compounds were identified in the methanol seed extract of R. indica. The spectra revealed the major compound present in seeds were Urs-12-en-3-ol, acetate (13.87 %), followed by (3á)- (CAS), 1-Methylbicyclo[2.2.1]heptan-7-one (10.25)%), Sibiricin (3.66 %) and (5E,7E)-5,7-dodecadien-1-ol (3.05 %) (Table1). Twenty nine compounds were identified in the methanolic root extract of Rorippa indica. The major bioactive compounds are 1-Iodo-9-octadecene (46.41 %), 4à-Isopropyl-7ámethyltetracyclo [8.4.0.0 (2, 7).0 (4, 6)] tetrad ca-1, 10-dien-12-one (17.71 %), and 9-Hexadecenoic acid (12.41 %) (Table 2). The phytocompounds and their biological activities obtained through the GC-MS analysis of R. indica seeds and roots were presented in the Table (3 and 4). n- Hexadecanioc acid ethyl ester and Octadecanoic acid have been reported in Pterocarpus marsupium ⁽¹²⁾ and Calophyllum inophyllum (13). The biological activities listed are based on Duke's Phytochemical and Ethonobotanical Database and Jim Duke Agricultural Research Service/ USDA.

Medicinal properties of Rorippa indica includes antiscorbutic, depurative, diuretic, expectorant, hypoglycemic, cancer preventive, odontalgic, purgative and stimulants⁽¹⁴⁾, of these properties, cancer preventive activity is confirmed by the bioactive compound octadecanoic acid from seed as well as root methanolic extracts Table (3 and 4). R. indica also have compounds with insecticidal activity like 5-Cyano-6-(2-thienyl)-3-(2-pyridyl)-1.2.4-triazine. 4-Benzoyl-3,5-dimethyl-1-(pnitrophenyl) pyrazole, 1-Methylbicyclo[2.2.1] heptan-7-one and 4-Benzoyl-3,5-dimethyl-1-(pnitrophenyl) pyrazole in seeds. Seed and root contains toxicity and pest control compounds like (2R,3S,4S,5R)-1-tert-Butyldiphenylsilyloxy-2,4dimethyl-3,5isopropylidenedioxyheptan-6-one,5-Cyano-6-(2-thienyl)-3-(2-pyridyl)-1,2,4-triazine, 1-Methylbicyclo [2.2.1]heptan-7-one, Ethane, isothiocyanato- (CAS) and ent-10á-Hydroxy-3á-(methoxymethyl)-16-oxo-17,20-dinor 9à,15àcyclogibberell-1-ene-7,19-dioic Acid 7-Methyl Este19,10-Lactone. Hexadecanoic acid (methyl ester) from root extract has anti-inflammatory property.

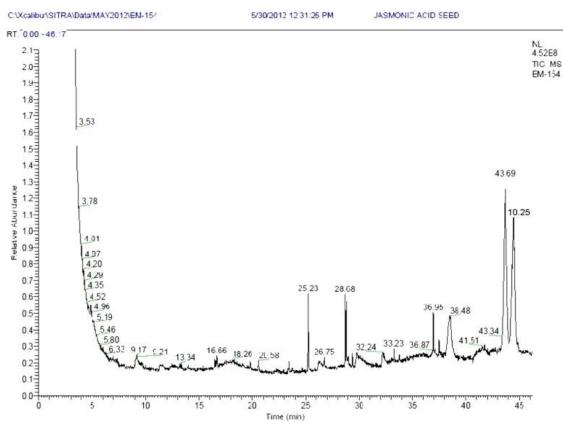
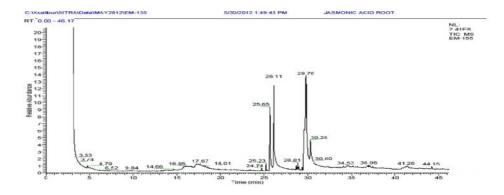


Figure 1: GC-MS analysis in methanolic elicitor treated seed extract of Rorippa indica L.

Figure 2: GC-MS analysis in methanolic elicitor treated root extract of Rorippa indica L.



S. No	*RT	Name of the Compounds	Molecular Formula	MW	Peak Area %
1	4.83	N,N,N'-trimethyl-N'- [(trimethylstannyl)methyl]ethylenediamine	$C_9H_{24}N_2Sn$	280	0.27
2	6.96	1,3,5-triazine-2,4,6(1H,3H,5H)-trione (CAS)	$C_3H_3N_3O_3$	129	0.38
3	7.34	TMS-1',8,11-tri-OH-tetrahydrocannabinol	$C_{33}H_{62}O_5Si_4$	650	0.15
4	9.15	(3S,4R)-3-(Mesyloxy)-4-hydroxytetrahydrofuran	$C_5H_{10}O_5S$	182	0.67
5	11.40	4,7-Dibromo-nido-5,6-dicarbaborane	$C_2H_{10}B_8Br_2$	280	0.38
6	12.47	(2R,3S,4S,5R)-1-tert-Butyldiphenylsilyloxy-2,4- dimethyl-3,5-isopropylidenedioxyheptan-6-one	$C_{28}H_{42}O_4Si$	470	0.23
7	13.34	3-Ethylthio-2-hydroxy-2-methyl-N-phenyl-1,4- dioxane-3-carboxamide	$C_{14}H_{19}NO_4S$	297	0.28
8	16.64	Benzene, 1-cyclobuten-1-yl- (CAS)	$C_{10}H_{10}$	130	0.54
9	20.58	Tetradecanoic acid, methyl ester (CAS)	$C_{15}H_{30}O_2$	242	0.28
10	22.24	3-Methoxypropenyl(phenyl)iodonium tetrafluoroborate	$C_{10}H_{12}BF_4IO$	362	0.19
11	23.44	5-Cyano-6-(2-thienyl)-3-(2-pyridyl)-1,2,4-triazine	$C_{13}H_7N_5S$	265	0.26
12	25.23	Eicosanoic acid, methyl ester (CAS)	$C_{21}H_{42}O_2$	326	1.80
13	26.26	Methyl 2-methoxy-5,5-dimethyltetrahydro-2-furoate	$C_9H_{16}O_4$	188	0.56
14	26.72	4-Benzyloxy-5-methoxy-1-methylindole-3-carboxylic acid	C ₁₈ H ₁₇ NO ₄	311	0.23
15	28.81	(5E,7E)-5,7-dodecadien-1-ol	$C_{12}H_{22}O$	182	3.05
16	29.38	meso-2,2,3,7,8,8-Hexamethyl-5-methylidenenonane- 3,7-diol	$C_{16}H_{32}O_2$	256	0.26
17	29.78	Decanoic acid (CAS)	$C_{10}H_{20}O_2$	172	0.85
18	32.24	p-Trimethylsilyl-à,alphaethylenedioxytoluene	$C_{12}H_{18}O_2Si$	222	0.85
19	33.25	9-Octadecenoic acid, methyl ester, (E)- (CAS)	$C_{19}H_{36}O_2$	296	0.39
20	36.95	4-Benzoyl-3,5-dimethyl-1-(p-nitrophenyl)pyrazole	$C_{18}H_{15}N_3O_3$	321	1.26
21	37.47	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]	C ₂₀ H ₄₀ O	296	0.50
22	38.50	Sibiricin	$C_{16}H_{18}O_5$	290	3.66
23	40.18	Azetidine (CAS)	C ₃ H ₇ N	57	0.17
24	41.21	N-(2',2'-Diethoxy-1'-ethyl)-4-[(pentafluorophenyl) oxycarbonyl]-3-phenyl-1-butyramide	$C_{23}H_{24}F_5NO_5$	489	0.23
25	41.74	1,6-Di2-phenylbenzimidazolylmethyl-2,5- piperazindione	$C_{32}H_{26}N_6O_2$	526	0.44
26	42.71	2,3-dicarbomethoxybenzoselenophene	$C_{12}H_{10}O_4Se$	298	0.54
27	43.69	Urs-12-en-3-ol, acetate, (3á)- (CAS)	C ₃₂ H ₅₂ O ₂	468	13.87
28	44.46	1-Methylbicyclo[2.2.1]heptan-7-one	$C_8H_{12}O$	124	10.25

Table1: Phytocompounds in methanolic extract of seed of *Rorippa indica* L.(Jasmonic acid treated)

*RT: Retention Time

 Table 2: Bioactivity in methanolic extract of seed of Rorippa indica L.(Jasmonic acid treated)

S. No	Name of the Compounds	Compound Nature	Biological Activity
	N,N,N'-trimethyl-N'- [(trimethylstannyl)methyl] ethylenediamine	Amine Compounds	Molluscide, fungicides and algaecide
2	1,3,5-triazine-2,4,6(1H,3H,5H)-trione (CAS)	Ketone Compound	Toxicity
3		Alcoholic Compound	Antimicrobial activity, sedatives and anesthetics
4	(3S,4R)-3-(Mesyloxy)-4-	-	No report

	hydroxytetrahydrofuran			
5	4,7-Dibromo-nido-5,6-dicarbaborane	Bromoide Compound	Acute toxicity, fumigants a Pesticide activity	
6	(2R,3S,4S,5R)-1-tert-Butyldiphenylsilyloxy- 2,4-dimethyl-3,5-isopropylidenedioxyheptan- 6-one	Ketone Compound	Toxicity	
7	3-Ethylthio-2-hydroxy-2-methyl-N-phenyl- 1,4-dioxane-3-carboxamide	Amide Compounds	No report	
8	Benzene, 1-cyclobuten-1-yl- (CAS)	-	No report	
9	Tetradecanoic acid, methyl ester (CAS)	Myristic acid Compound	No report	
10	3-Methoxypropenyl(phenyl)iodonium tetrafluoroborate	-	No report	
11	5-Cyano-6-(2-thienyl)-3-(2-pyridyl)-1,2,4- triazine	Cyanoide Compound	Pest control	
12	Eicosanoic acid, methyl ester (CAS)	Arachidic acid Compound	Detergents, photographic materials and lubricants	
13	Methyl 2-methoxy-5,5-dimethyltetrahydro-2- furoate	-	No report	
14	4-Benzyloxy-5-methoxy-1-methylindole-3- carboxylic acid	Acid Compound	No report	
15	(5E,7E)-5,7-dodecadien-1-ol	Alcoholic Compound	Antimicrobial activity, sedative and anesthetics	
16	meso-2,2,3,7,8,8-Hexamethyl-5- methylidenenonane-3,7-diol	Alcoholic Compound	Antimicrobial activity, sedatives and anesthetics	
17	Decanoic acid (CAS)	Fatty acid Compound	No report	
18	p-Trimethylsilyl-à,alpha ethylenedioxytoluene	-	No report	
19	9-Octadecenoic acid, methyl ester, (E)- (CAS)	Stearic acid Compound	No report	
20	4-Benzoyl-3,5-dimethyl-1-(p-nitrophenyl) pyrazole	Nitro Compound	Fungicide, insecticide and herbicide	
21	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R[R*,R*-(E)]]	Alcoholic Compound	Antimicrobial activity, sedatives and anesthetics	
22	Sibiricin	-	No report	
23	Azetidine (CAS)	Amine Compound	Antihypertensive, antiinflammatory, antiarrhythmic, antidepressant and Monoamine oxidase inhibitory activities.	
24	N-(2',2'-Diethoxy-1'-ethyl) [(pentafluorophenyl) oxycarbon yl] -3-phenyl-1-butyramide	Amide Compound	No report	
25	1,6-Di2-phenylbenzimidazolylmethyl-2,5 piperazindione	Ketone Compound	Toxicity	
26	2,3-dicarbomethoxybenzoselenophene	-	No report	
27	Urs-12-en-3-ol, acetate, (3á)- (CAS)	Alcoholic Compound	Antimicrobial activity, sedatives, and anesthetics	
28	1-Methylbicyclo[2.2.1] heptan-7-one	Ketone Compound	Toxicity and pesticidal activity	

S. No	*RT	Name of the Compounds	Molecular Formula	MW	Peak Area %
1	4.79	Butanal (CAS)	C ₄ H ₈ O	72	0.42
2	9.69	Piperidin-4-carboxylic acid	$C_6H_{11}NO_2$	129	0.23
3	13.57	3,3'-Anhydro-3-C-(3-hydroxymethyl)-1,2;5,6-di-O- isopropylidene-à,D-allofuranose	$C_{13}H_{20}O_6$	272	0.12
4	14.66	(Tricyclo[4.4.1.0(1,6)]undeca-3,8-dien-11,11- dimethyl)sulfiTe	$C_{13}H_{16}O_3S$	252	0.16
5	15.84	3-Chloromethyl-3-methoxymethyl-oxacyclo butane	$C_6H_{11}ClO_2$	150 3	0.76
6	16.62	2,6-Dihydroxy-3,5-dimethoxy-N,N-dimethyl- tetrahydro-10H-naphtho[1,2,3-ij]isoquinoline	$C_{20}H_{23}NO_4$	341	0.49
7	17.29	tert-butylacetyl chloride-(18)O	C ₆ H ₁₁ ClO	134	1.99
8	18.22	2-(4-Methylphenyl)-4-formylquinoline	$C_{17}H_{13}NO_2$	263	0.12
9	20.56	1,3-dimethoxy-9h-xanthene	$C_{15}H_{14}O_3$	242	0.10
10	23.40	Ethane, isothiocyanato- (CAS)	C ₃ H ₅ NS	87	0.18
11	24.74	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis- (CAS)	$C_{19}H_{36}O_3$	312	0.30
12	25.23	Hexadecanoic acid, methyl ester (CAS)	$C_{17}H_{34}O_2$	270	1.00
13	25.65	9-Hexadecenoic acid (CAS)	$C_{16}H_{30}O_2$	254	12.14
14	26.11	4à-Isopropyl-7á-methyltetracyclo[8.4.0.0(2,7) .0(4,6)]tetradeca-1,10-dien-12-one	C ₁₈ H ₂₄ O	256	17.17
15	28.81	9-Octadecenoic acid (Z)-, methyl ester (CAS)	$C_{19}H_{36}O_2$	296	1.59
16	29.78	1-Iodo-9-octadecene	C18H ₃₅ I	378	46.41
17	30.20	Octadecanoic acid (CAS)	C ₁₈ H ₃₆ O ₂	284	3.92
18	32.28	Nitrous acid, cyclohexyl ester (CAS)	$C_6H_{11}NO_2$	129	0.38
19	34.09	N-Heptylhexanamide	C ₁₃ H ₂₇ NO	213	0.15
20	34.55	rel-(1R,4aS,8aS)-4-methyl-4a,5,6,7,8,8a-hexa hydro-1H-2,3-benzoxazine-3-oxide	C ₉ H ₁₅ NO ₂	169	0.44
21	37.11	Oxirane, hexyl- (CAS)	C ₈ H ₁₆ O	128	1.48
22	37.45	anti-5-(1-hydroxyundecyl)-3-(3-methylbutyl) oxazolidin-2-one	C ₁₉ H ₃₇ NO ₃	327	0.15
23	38.48	butyl(3aà,4à,5à,6aà)-4-(dimethoxymethyl)-3a,5, 6,6a-tetrahydro-5 -hydroxy-2-(4-methoxy carbonylbutyl)-4H-cyclopenta[b]furan-3- carboxylate	$C_{21}H_{34}O_8$	414	0.13
24	40.80	1-Amino-7-methylpyrrolo[1,2-a]pyrazine	$C_8H_9N_3$	147	0.16
25	41.26	Octane, 2-bromo- (CAS)	C ₈ H ₁₇ Br	192	0.70
26	42.74	ent-10á-Hydroxy-3á-(methoxymethyl)-16-oxo- 17,20-dinor 9à,15à-cyclogibberell-1-ene-7,19-dioic Acid 7-Methyl Este19,10-Lactone	$C_{21}H_{24}O_7$	388	0.11
27	43.58	(3S*,2aS*,9bS*,9aS*)-5a-aza-2,2a,3,5,6,7,8,9,9a, 9b-decahydro-3-methyl-5-oxo-4-oxa-1H-benz [c,d]azulene	C ₁₂ H ₁₉ NO ₂	209	0.11
28	44.15	(1S,2S,4R)-8-p-Menthene-1,2-diol	$C_{10}H_{18}O_2$	170	0.40
29	44.89	Anhydro-illiensine	C ₂₄ H ₃₇ NO ₆	435	0.14

 Table 3: Phytocompounds in methanolic extract of root of Rorippa indica L.(Jasmonic acid treated)

*RT: Retention Time

S. No	Name of the compounds	Compound Nature	Biological activity No report	
1	Piperidin-4-carboxylic acid	-		
2	3,3'-Anhydro-3-C-(3-hydroxymethyl)-1,2;5,6-di-O- isopropylidene-à,D-allofuranose	-	No report	
3	(Tricyclo[4.4.1.0(1,6)]undeca-3,8-dien-11,11- dimethyl)sulfite	Sulfur compound	Antimicrobial	
4	3-Chloromethyl-3-methoxymethyl-oxacyclobutane	-	No report	
5	2,6-Dihydroxy-3,5-dimethoxy-N,N-dimethyl- tetrahydro-10 H-naphtho[1,2,3-ij]isoquinoline	-	No report	
6	Tert-butylacetyl chloride-(18)	Chloride compound	No report	
7	2-(4-Methylphenyl)-4-formylquinoline	-	No report	
8	1,3-dimethoxy-9h-xanthene	-	No report	
9	Ethane, isothiocyanato- (CAS)	Cyanoide Compound	Pest control	
10	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis- (CAS)	-	No report	
11	Hexadecanoic acid, methyl ester (CAS)	Ester Compound	Anti inflammatory Fragrances	
12	9-Hexadecenoic acid (CAS)	-	No report	
13	4à-Isopropyl-7á-methyltetracyclo [8.4.0.0(2,7). 0(4,6)] tetradeca-1,10-dien-12-one	Ketone Compound	Toxicity	
14	9-Octadecenoic acid (Z)-, methyl ester (CAS)	Stearic acid Compound	No report	
15	1-Iodo-9-octadecene	-	No report	
16	Octadecanoic acid (CAS)	-	No report	
17	Nitrous acid, cyclohexyl ester (CAS)	Nitro compound	No report	
18	N-Heptylhexanamide	-	No report	
19	rel-(1R,4aS,8aS)-4-methyl-4a,5,6,7,8,8a-hexahydro- 1H-2,3-benzoxazine-3-oxide	-	No report	
20	Oxirane, hexyl- (CAS)	-	No report	
21	anti-5-(1-hydroxyundecyl)-3-(3-methylbutyl)oxa zolidin-2-one	Ketone Compound	Toxicity	
22	butyl(3aà,4à,5à,6aà)-4-(dimethoxymethyl)-3a,5,6,6a- tetrahydro-5-hydroxy-2-(4-methoxycarbonylbutyl)- 4H-cyclopenta[b] furan-3-carboxylate	-	No report	
23	1-Amino-7-methylpyrrolo[1,2-a]pyrazine	-	No report	
24	Octane, 2-bromo- (CAS)	Broamide Compound	No report	
25	ent-10á-Hydroxy-3á-(methoxymethyl)-16-oxo-17,20- dinor 9à,15à-cyclogibberell-1-ene-7,19-dioic Acid 7- Methyl Este19,10-Lactone	Ketone Compound	Toxicity	
26	(3S*,2aS*,9bS*,9aS*)-5a-aza-2,2a,3,5,6,7,8,9,9a,9b- decahydro-3-methyl-5-oxo-4-oxa-1H-benz[c,d] azulene	-	No report	
27	(1S,2S,4R)-8-p-Menthene-1,2-diol	Alcoholic Compound	No report	
28	Anhydro-Illiensine	-	No report	

Table 4: Bioactivity in methanolic extract of root of *Rorippa indica* L. (Jasmonic acid treated)

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