22. Bioactive potentials of Inonotus Rickii (Pat.) D.A. Reid from Pune

Neeta Jagtap[1], Kiran Ranadive[2], Pradnya Jagtap[3], Nayana Chaudhari[4], Priya Thopate[5], Priyanka Jagtap[6] and Snehal Salunke[7]

[1, 4,5, 6&7]Department of Chemistry, P.D.E.A.'s Waghire College, Saswad, Taluka Purandar, District-Pune.

[2]Department of Botany, P.D.E.A.'s Waghire College, Saswad, Taluka Purandar, District-Pune.

[3]Department of Pharmacology, P.D.E.A.'s SGRS College, Saswad, Taluka Purandar, District-Pune.

Abstract

Inonotus is belongs to Aphyllophorales (Group of wood rotting fungi). It's also considered as a severe rot of the standing tree flora of road side as well as forests. More than 265 records are there of the same on Index Fungorum from which 15 are reported so far from India.This is the first attempt to do the different phytochemical tests of the same fungus. The tests showed very significant results like presence reducing sugars, steroids, flavonoids, monosaccharideand saponin glycosides.

Key words: Aphyllophorales, Basidiomycetes,*Inonotus*, Fungi

Introduction:

Aphyllophorales play very important role in the recycling process. *Inonotus* is one of the fungus from the same group of Basidiomycetous fungi. The family Hymenochaetaceae includes more than 34 genera like *Phellinus*, *Hymenochaete* etc. *Inonotus* is one of the most common disease of the tree flora along the road side trees and in the dense forest patches. This genus includes more than 265 species records from World (Index Fungorum 2018). In India there are only 15 reports of the different species. Many a times the fruit body is having anamorphic stage and sometimes may get the telomorphic stage at the same place.

Inonotusrickii(Pat.) D.A. Reid, Kew Bulletin 12 (1): 141 (1957)

In the present species chlamydospores are abundant in context tissue, thick-walled, dark reddish brown in KOH, negative in Melzer's reagent, irregular in shape, smooth, globose to ellipsoid or often with an elongated cylindric appendage, 14.985 X 12.321µm thick. It was found

on dead tree hardwoods and also parasites of *Delonixregia.*The distribution of the same species is wide in United States (Florida, Louisiana, and Arizona) and only in the ptychogastric stage. Its true distribution in the Neotropics is unknown but widespread in the tropics.

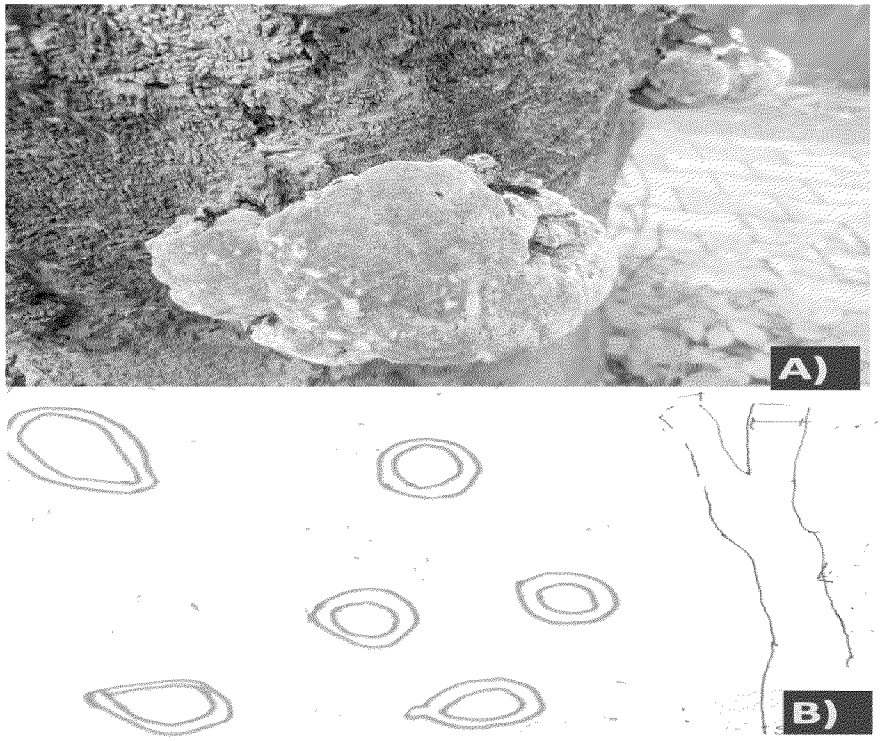

Fig.01 A)*Inonotus rickii*: B) a. Conidia (14.985 X 12.321µm) and Sterile hypha (8.325 µm)

Materials and Methods:

Collection and Preservation of the sample

Collection: The sample of*Inonotus*was collected from Pune with sample code WCSKR-02. The specimen was kept in the specimen folder. The crude powder*Inonotus* was then preserved by the standard method.**Preservation of samples:**During the collection tours the fungal material, mostly dead twigs and wood with fruit bodies were examined using a 3 fold pocket lens (15X) and kept in the zip lock polythene bags as well as some times paper bags. Most of the forms collected were growing saprophytically and their substrates were identified following the latest nomenclature after tentative identification on the spot. The specimen was brought to the laboratory and examined under a stereo-binocular to observe position of the fruit bodies, their gross morphology, shape, size, colour of the fruit body, presence or absence of the appendages etc.

The specimen was labeled and deposited in the Department of Botany, Waghire College, Saswad, Taluka-Purandar, District- Pune, Pin-412301 with the WCSKR-02 accession number.The collected material was kept in brown paper folders of size 20 x 15 cms prepared from paper of 29 x 33 cms size with label (Size 16.5 x 7.5 cms) having details:**Photographic Documentation:**The specimen was photographed with the help of Nikon 3200 (SLR) zoom lens camera to get the best result showing all Macro-morphological details of the specimen. Selection of the quality photographs was done by checking its zooming quality. **Camera Lucida Drawing Preparation:**Camera lucida sketches were also made for all materials with mirror type camera lucida. Measurements of the morpho-taxonomic structures like sterile hyphae, spores were made by ocular of ERMA INC, made in Japan and objective micrometer of ERMA TOKYO company and eyepiece micrometer under 10X, 45X and 100X objectives. For fructification, measurements were taken 5-10 times and average values were recorded.

Identification: Materials were identified up to species level with the aid of standard literature namely Donk MA. (1933), Fiasson JL, Niemela T. (1984) , Gilbertson RL. (1976), Gilbertson RL, Ryvarden L. (1986), Gilbertson RL, Wright JE, Monclavo, JM.(2002), Kulkarni S. K. (2012), Pegler DN. (1964) ,Sharma JR, Das K and Mishra D. (2013), Rayner RW. (1970) , Ryvarden L. (1991),Ryvarden L. (2005) ,/www.fungifromindia.com, www.indexfungorum.org, and www.mycobank.org

Procedure of extraction:

The sample was extracted from the spore powder in different solvent system like water, Ether and Ethanol. All air and sundried samples were subjected to the standard extraction procedure using Soxlet apparatus. The extracted samples were then stored in a bottles. The extracted samples then concentrated by using the simple heating process. These concentrated samples were used for testing the Phytochemical tests as follows.(**Table No. 01, 02 & 03**)

Table No.01 Phytochemical test of *Inonotus*in different solvent systems

Sr. No	TEST	Crude (*Inonotus*powder)		Ether *Inonotus*		Ethanol *Inonotus*		Water *Inonotus*	
		Observation	Inference	Observation	Inference	Observation	Inference	Observation	Inference
	Test for Carbohydrates								
A)	**Test for Reducing sugars**								
1)	**Fehlings test**								
	1 ml Fehling A and 1 ml Fehlings B soln boil .add equal volume of test soln .heat in boiling water bath for 10 min	First yellow then brick ppt is observed	Reducing sugar may be present	No ppt.	Reducing sugar absent	No ppt.	Reducing sugar absent	First yellow then brick ppt is observed	Reducing sugar may be present
2)	**Bendict test** Equal volume of bendicts reagent + test soln.heat in boiling water bath for 5 min soln appears green, yellow red	Red colour solution	Reducing sugar may be present	Red colour solution	Reducing sugar may be present	Red colour solution	Reducing sugar may be present	Red colour solution	Reducing sugar may be present
B)	**Test for Monosaccharides**								
1)	**Barfoeds test** Mix equall volume of barfoeds reagent & test reagent. heat for 1-2 min in boiling water bath &cool . Red ppt observed	Red ppt is observed	Monosaccharide may be present	No red ppt	monosaccharides absent	Red ppt is observed	Monosaccharide may be present	Red ppt is observed	Monosaccharide may be present
C)	**Test for Pentose sugars**								
1)	Mix equall amount of test soln& HCl. Heat this mix .add	Red colours is observed appears	Pentose sugar may be present	Red colour appear	Pentose sugar may be present	No red colour appears	Pentose sugar absent	Red colour appear	Pentose sugar may be present
	a crystal of pholoroglucilnol . Red color appears								
D)	**Test for Hexose sugars**								
1)	Tollens phloroglucinol test for galactose mix 2.5ml conc Hcl & 4ml 0.5ml phloroglucinol+1-2ml test soln heat . Yellow red colour .	No yellow to red colour observed	Hexose sugar absent	No yellow to red colour observed	Hexose sugar absent	Yellow to red colour observed	Hexose sugar present	Yellow to red colour observed	Hexose sugar present

E)	**Test for Non-Reducing sugars**								
	Test soln does not give response to fehling & benedict test	Give respons to fehlings & bendict test	Non reducing sugar absent	Give response to fehling & bendict test	Non reducing sugar absent	Give response to fehling & bendict test	Non reducing sugar absent	Give response to fehling & bendict test	Non reducing sugar absent
F)	**Test for non-Reducing Polysaccharides (starch)**								
1)	**Iodine test** 3ml test soln +few dropes of dilute iodine soln	Blue colour does not appears	Non reducing polysaccharides absent	Blue colour does not appears	Non reducing polysaccharides absent	Blue colour does not appears	Non reducing polysaccharides absent	Blue colour does not appears	Non reducing polysaccharides absent
	Test for proteins								
1)	**Biuret test** 3ml test soln +4% NaoH &few dropes of 1% CuSo4 soln violet or pink colour appear	No violet pink colour observed	Proteins absent	No violet pink colour observed	Proteins absent	No violet pink colour observed	Proteins absent	No violet pink colour observed	Proteins absent
2)	**Million test** 3ml test soln + 5ml millon soln on reagent	No white ppt	Proteins absent	No white ppt	Proteins absent	No white ppt	Proteins absent	No white ppt	Proteins absent
	Test for amino acids								
1)	**Ninhydrin test**								
	3ml test soln +3 droppes 5% ninhydrin soln. heat in boiling water bath for 10min .Purpal or bluish colour appears	No purple or bluish colour appear	Amino acid absent	No purple or bluish colour appear	Amino acid absent	No purple or bluish colour appear	Amino acid absent	No purple or bluish colour appear	Amino acid absent
2)	**Test for Cysteine** 5ml test soln + few dropes of 40% NaoH +10% lead acetate soln.boil the soln black ppt of lead sulphat is formed	No black ppt	Amino acid absent	No black ppt	Amino acid absent	No black ppt	Amino acid absent	No black ppt	Amino acid absent
	Test for steroids								
1)	**Salkowski reaction**								
	2ml extract + 2ml chloroform + 2ml conc.H_2SO_4. Shake well .chloroform layer	No colouration	Steroid absent	No colouration	Steroid absent	Chloroform layer appears red & acid layer shows greenish	Steroid may be present	No colouration	Steroid absent

	appears red and acid layer shows greenish yellow fluorescence					fluroscence			
	Test for Glycosides								
A)	**Test for Cardiac Glycosides**								
1)	**Test for Deoxy-sugars** 2 ml extracts + glacial acetic acid + 1 drop 5% $feCl_3$ + conc. H_2SO_4 redish brown colour appear at the junction of two liquid layer and upper layer appears bluish green	No colouration	Cardiac glycoside absent	No colouration	Cardiac glycoside absent	No colouration	Cardiac glycoside absent	No colouration	Cardiac glycoside absent
B)	**Test for Saponin Glycoside**								
1)	**Foam test** Shake the drug extract or dry powder vigorously with water.persistant foam observed	No persistent foam observed	Saponin glycoside absent	No persistent foam observed	Saponin glycoside absent	No persistent foam observed	Saponin glycoside absent	Persistent foam is observed	Saponin glycoside may be present
C)	**Test for Cynogenetic Glycosides**								
1)	**Grignard reaction or Sodium Picrate test** soak a filter paper strip first in 10% picric acid, then in 10% sodium carbonate, dry. In conical flask place moistened powdered drug. Cork it , place it above filter in slit in cork the filter paper turns brick red or maroon.	No change obeserved on filter paper	Cynogenetic glycoside absent	No change obeserved on filter paper	Cynogenetic glycoside absent	No change obeserved on filter paper	Cynogenetic glycoside absent	No change obeserved on filter paper	Cynogenetic glycoside absent
1)	**Dragendroff's test** 2-3ml filtrate+few drops Dragendroff's reagent. Orange	no ppt formed	Alkaloids absent	No ppt formed	Alkaloids absent	No ppt formed	Alkaloids absent	No ppt formed	Alkaloids absent

	brown ppt is formed								
2)	**Mayer's test** 2ml filtrate +Mayer's reagents gives ppt.	no ppt	Alkaloid absent	No ppt	Alkaloid absent	No ppt	Alkaloid absent	No ppt	Alkaloid absent
Test for Tannins & Phenolic compounds									
1)	**Lead Acetate test** 2-3ml lead acetate test solution +test solution gives white ppt.	No ppt	Tannins & phenolic compounds absent	No ppt	Tannins & phenolic compounds absent	No ppt	Tannins & phenolic compounds absent	No ppt	Tannins & phenolic compounds absent
2)	**Bromine water test** 2-3 ml test solution +bromine water. Decolourisation of bromine water	No decolourisation	Tannins & phenolic compounds absent	No decolourisation	Tannins & phenolic compounds absent	No decolourisation	Tannins & phenolic compounds absent	No decolourisation	Tannins & phenolic compounds absent
Test for Flavonoids									
1)	**H_2SO_4 Test**On addition of H_2SO_4flavanones & flavanodes dissolve into it & gives a deep yellow solution .Chalcones & aurones gives red or red-bluish solution.Flavanes give orange to red colour.	No colourisation	Flavonoids absent	No colourisation	Flavonoids absent	Orange to red colour	Flavonoids may be present	Deep yellow colour	Flavonoids may be present
2)	**Lead Acetate test** Small amount of residue + lead acetate solution. Yellow ppt.	No yellow ppt.	Flavonoids absent	No yellow ppt.	Flavonoids absent	Yellow ppt. is observed	Flavonoids may be present	No yellow ppt.	Flavonoids absent
1)	**Catalase** To thick sections of drug, H_2O_2, oxygen gas evolves	O_2 gas does not evolved	Catalase absent	O2 gas does not evolved	Catalase absent	O2 gas does not evolved	Catalase absent	O2 gas does not evolved	Catalase absent
Test for vitamins									
1)	**Test for vitamins C (Ascorbic acid)** To 2 ml of 2% w/v solution add 2ml of water, 0.1 gm sodium bicarbonate and	Deep violet colour absent	Ascorbic acid may be absent	Deep violet colour absent	Ascorbic acid may be absent	Deep violet colour absent	Ascorbic acid may be absent	Deep violet colour absent	Ascorbic acid may be absent

	about 20 mg ferrous sulphate. Shake and allow to stand; deep violet colour is produce. Add 5ml of 1 M sulphuric acid. Colour dissapear	nt							

Table No:02 Phytochemical conclusion

Sr.no	Test	Solvent used
1	Reducing Sugars	Ether, Ethanol, Water, Crude powder
2	Monosaccharide	Water, Ethanol, Crude powder
3	Steroids	Ethanol
4	Saponin glycosides	Water
5	Flavanoids	Ethanol, Water

Table No. 03 Phytochemical conclusion:-

Extracts	No. of Phytochemical constituents
Ether	1
Ethanol	4
Water	4
Crude powder	2

Figure no: 4 No. of phytochemical constituents

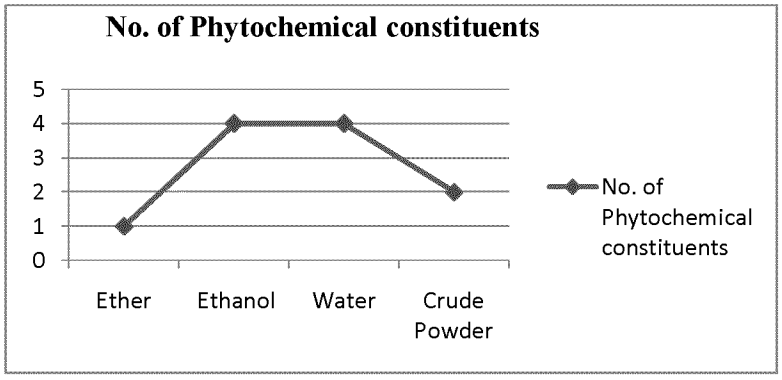

Fig.02 No. of Phyto-constituents in *Inonotus rickii*

Result and conclusions:

The **Ether extract**:-In ether extracts of *Inonotus* one phytochemical constituents i.e. reducing sugar is present.**Ethanol extract**:-In ethanol extract of *Inonotus* four phytochemical

constituents i.e. reducing sugar, steroids, Flavonoids and Monosaccaride are present.**Water extract** :-In water extract of *Inonotus* four phytochemical constituents i.e.reducing sugar, monosaccarides, Flavonoids and Saponin Glycosides. The crude sample of *Inonotus*two phytochemical constituents i.e. Reducing sugar and Monosaccharides. Many more such hidden novel properties may be studied like this by different protocols.

Acknowledgement :

We are very much thankful to Hon. Principal **Dr. N.L.Ghorpade**, Waghire College Saswad and Hon. Principal **Dr. R.Y. Patil**, SGRS College of Pharmacy, for their kind permission to conduct the experiments for this work. At the outset we would like to thank **Prof. Dhorge S.M**. Head Department of Chemistry Waghire college Saswad,**Prof. Ganesh Nigade** and **Prof. Vaibhav Shilimkar from SGRS college of Pharmacy Saswad,** for their valuable guidance during this work. We would like to express our deep sense of gratitude towards **Miss. Bhagyashree Vyapari, Miss. Prachali Chavan, Miss. Bhakti Lonkar** and **Miss. Pooja Pisal** for the actual experimentation work in the lab**.** We would like to extend our sincere thanks to all the staff members of Department of Chemistry and our Lab Assistant **Mr. Ganesh Bhosale** for providing help during our project work. We are happy to take this opportunity to express our gratitude to those who have been helpful to us in completing this project report. Lastly, we would like to thank our parents, friends and well-wishers who have encouraged us to do this research work and all those who contributed directly or indirectly in completing this project to which we are obliged.

References :-

1. **Donk MA.** (1933) Revision der Niederlandischen *Homobasidiomycete. Aphyllophoraceae*. Meded. Bot. Mus. Herb. Utrecht 9: 1-278.
2. **Fiasson JL, Niemela T.** (1984) The Hymenochaetales: a revision of the European poroid taxa. Karstenia 24, 14–28.
3. **Gilbertson RL.** (1976) The genus *Inonotus* (Aphyllophorales, Hymenochaetaceae) in Arizona. Mem. N.Y. Bot. Gard. 28, 67–85.
4. **Gilbertson RL, Ryvarden L.** (1986) North American Polypores-1, Fungiflora, Oslo. 434 p.

5. **Gilbertson RL, Wright JE, Monclavo, JM.**(2002) *Inonotus* s.l. in Argentina. Morphology, cultural characters and molecular analyses. Mycological Progress 1, 299–313.
6. **Kulkarni S. K.** (2012) Handbook Of Experimental Pharmacology, Vallabh Prakashan.
7. **Pegler DN.** (1964) A survey of the genus *Inonotus* (Polyporaceae). Transactions of the British Mycological Society 47(2), 175–195.
8. **Ranadive, K.R.** (2014) Eight new records of poroid fungi from Western Ghats of Pune district (Maharashtra) Kavaka 42:29-33.
9. **Ranadive K.R., Jite P.K., Ranade V.D., Vaidya J.G.**(2013) Flora of Aphyllophorales from Pune district - part I . Journal on New Biological Reports 2(3): 188-227 (2013)
10. **Sharma JR, Das K and Mishra D.**(2013) The genus *Inonotus* and its related species in India. Mycosphere 4(4), 809–818, Doi 10.5943/mycosphere/4/4/16
11. **Rayner RW.**(1970) A mycological colour chart. Commonwealth Mycological Institute, Kew, 9 plates, 34 p.
12. **Ryvarden L.**(1991) Genera of polypores. Nomenclature and taxonomy. Fungiflora, Oslo. 363 p.
13. **Ryvarden L.** (2005) The genus *Inonotus*, a synopsis. Synopsis Fungorum 21, 149 p.
14. http://www.fungifromindia.com/fungiFromIndia/buildPage.php?page=home
15. http://www.indexfungorum.org/names/names.asp
16. http://www.mycobank.org/defaultinfo.aspx?page=home